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Effects of experimental otter trawling on the macrofauna of a sandy bottom ecosystem on the Grand Banks of Newfoundland

E.L.R. Kenchington, J. Prena, K.D. Gilkinson, D.C. Gordon, Jr., K. MacIsaac, C. Bourbonnais, P.J. Schwinghamer, T.W. Rowell, D.L. McKeown, and W.P. Vass

Abstract: A 3-year otter trawling experiment was conducted on a deepwater (120–146 m) sandy bottom ecosystem on the Grand Banks of Newfoundland that had not experienced trawling for at least 12 years. The benthic macrofauna were sampled before and after trawling and in reference areas. The 200 grab samples collected contained 246 taxa, primarily polychaetes, crustaceans, echinoderms, and molluscs. Biomass was dominated by propeller clams (*Cyrtodaria siliqua*) and sand dollars (*Echinarachnius parma*), while abundance was dominated by the polychaete *Prionospio steenstrupi* and the mollusc *Macoma calcarea*. The most prominent feature of the data was a natural decline in the total number of species, the total abundance, and the abundance and biomass of selected species between 1993 and 1995. The only immediate effect of trawling was seen in 1994 when the abundance of 13 species, the biomass of 11 species (mostly polychaetes), and the total abundance per grab were significantly lower. There was little evidence of long-term trawling effects. When trawling disturbance was indicated, it appeared to mimic natural disturbance, shifting the community in the same direction in multidimensional scaling ordination; no distinctive trawling signature was observed. However, the results of this experiment should not be uncritically extrapolated to the impacts of commercial trawling.

Résumé : Pendant 3 ans, la pêche au chalut à panneaux a été pratiquée dans un écosystème profond (120–146 m) à fond sablonneux sur les Grands bancs de Terre-Neuve où il n'y avait pas eu de chalutage depuis au moins 12 ans. Des récoltes de la macrofaune benthique ont été faites avant et après le chalutage, ainsi que dans des zones témoins. Les 200 échantillons récoltés à la benne contenaient 246 taxons, principalement des polychètes, des crustacés, des échinodermes et des mollusques. Les Pitots (*Cyrtodaria siliqua*) et les Oursins plats de l'Atlantique (*Echinarachnius parma*) dominaient la biomasse, alors que le polychète *Prionospio steenstrupi* et le mollusque *Macoma calcarea* étaient les taxons les plus abondants. La caractéristique la plus évidente qui ressort de ces données est l'existence d'un déclin naturel dans le nombre d'espèces, la densité totale et l'abondance et la biomasse d'espèces particulières entre 1993 et 1995. Le seul effet immédiat du chalutage est apparu en 1994 lorsque la densité de 13 espèces et la biomasse de 11 espèces, surtout des polychètes, ainsi que la densité totale par échantillon de benne étaient significativement plus basses. Il y a peu de signes d'effets à long terme du chalutage. Lorsqu'il y a une indication de perturbation, il s'agit d'un phénomène qui mime les perturbations naturelles et qui se reflète par un glissement de la communauté dans la même direction sur une ordination de cadrage multidimensionnel; aucune signature particulière reliée au chalutage n'est décelée. Néanmoins, les résultats de notre expérience ne doivent pas être extrapolés de façon non critique dans l'évaluation des impacts du chalutage commercial.

[Traduit par la Rédaction]

Introduction

Mobile fishing gear such as beam trawls, otter trawls, scallop rakes, and clam dredges are widely used around the world to harvest fishery resources. Growing concern has been expressed over the potential effects of this gear on habitat, benthic communities, and fisheries. This global issue has stimulated scientific studies that have been summarized in recent reviews (e.g., Auster and Langton 1999; Hall 1999; Collie et al. 2000). It is clear that mobile fishing gear can

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cause environmental effects, but their magnitude depends on many factors, including the type of gear, intensity and distribution of use, and the kinds of benthic habitat and communities impacted.

Various approaches have been taken to study these fishing gear impacts. Small-scale, controlled experiments can provide direct evidence of immediate impacts (e.g., Currie and Parry 1996; Tuck et al. 1998), but, to date, these have only been conducted in relatively shallow water (where wave-generated natural disturbances frequently affect the seabed), have been of limited spatial extent, and have been short term in nature. Experiments on fishing effects of the extensive fisheries in the open sea are much more difficult to conduct (Hall 1994). Alternatively, observational studies comparing areas with different fishing histories can provide indirect evidence of longer-term impacts at the spatial scales of whole fishing grounds (e.g., Auster et al. 1996; Collie et al. 1997; Thrush et al. 1998), although the conclusions may be equivocal, as natural fluctuations may be mistakenly attributed to human impacts.

Over the past 50 years, otter trawls have been the primary mobile groundfish gear used on Canada's Atlantic continental shelf (Messieh et al. 1991). However, it is only very recently that a concerted effort has been made to investigate the environmental impacts associated with otter trawling in this region (Gordon et al. 1998). That effort has coincided with increasing concern over the potential environmental impacts of otter trawling and debate over the possible role of trawling impacts in the collapse of regional groundfish stocks in the early 1990s (Hutchings 1996). Although otter trawls are generally regarded as less damaging than other mobile gear types (Collie et al. 2000), they are still capable of inflicting physical and biological disturbance (e.g., Rumohr and Krost 1991; Service and Magorrian 1997; Engel and Kvitek 1998).

In order to obtain more detailed information on the effects of otter trawling on the benthic habitats and communities of offshore fishing grounds, the Department of Fisheries and Oceans conducted a 3-year (1993–1995) experiment on the Grand Banks of Newfoundland. The basic questions addressed were (*i*) can the effects of otter trawling be detected in this offshore environment in the face of inherent natural variability and, if so, (*ii*) could recovery be seen within 1 year. This experiment was different from most previous otter trawl impact studies in that it was conducted in deep water (120–146 m), it employed replicate experimental corridors, and the trawling disturbance was repeated 3 years in a row. This design allowed us to investigate both the immediate and longer-term effects of otter trawling on a bottom typical of large areas of the Grand Banks. The effects of the otter trawling on habitat structure at the experimental site have been reported elsewhere (Schwinghamer et al. 1996, 1998). The effects of the experimental trawling on the megaepibenthic community at this site, as sampled with an epibenthic sled, have been reported by Prena et al. (1999) and demonstrated that the biomass of snow crabs, sand dollars, soft corals, sea urchins, and brittle stars was significantly lower after trawling. This paper reports the effects of the trawling on the macrofaunal community as sampled by a large videograb. Megaepibenthic species captured by the grab are also included in the analyses.

Materials and methods

Experimental site

The 20 × 20 km experimental site is located on the northeastern part of Grand Bank, centred at 47°10′N, 48°17′W (Fig. 1). Exploratory sampling in 1992 demonstrated that the benthic community at this location had a relatively high number and biomass of epibenthic and infaunal species and a relatively homogeneous species composition (Prena et al. 1996). The depth range is 120–146 m and surficial sediments are moderately to well-sorted fine- to medium-grained sands (Schwinghamer et al. 1998), similar to those found fairly widely around the perimeter of Grand Bank and elsewhere in the northwest Atlantic (Fader and Miller 1986). The seabed at this site is reported to be relatively stable with no evidence of wave-induced ripples (Barrie et al. 1984), although interannual variations in sediment grain size and acoustic properties were observed during the experiment (Schwinghamer et al. 1998). Hard-bottom biogenic structures, such as mussel reefs, are not present and sessile epifaunal species occur only at low densities. The patchiness of epibenthic megafauna in this area has been described by Schneider et al. (1987).

Until the early 1990s, the fishery in this general region of the Grand Bank was for mixed groundfish, mostly American plaice (*Hippoglossoides platessoides*) with some Atlantic cod (*Gadus morhua*). Analysis of fishing effort records indicates, however, that the experimental site and its immediate surrounds had not been fished since the early 1980s (Kulka 1991; Prena et al. 1999). The main reason for this relocation of effort was the marketing strategy adopted by the large fish processors. Rather than being limited to harvesting primarily American plaice in the area of the experimental site, the fishing fleets targeted mixed groundfish further to the west (Kulka 1991).

In 1992, the Department of Fisheries and Oceans formally closed the experimental site to all commercial fishing activity for the duration of the experiment. In addition, the cod fishery in the North Atlantic Fisheries Organization Divisions 2J3KL, including the experimental site, was put under a fishing moratorium that same year. In 1993, most other groundfish fisheries on the Grand Bank were also closed. Sidescan sonar information collected during the experiment (Schwinghamer et al. 1998) confirmed that the only trawling that took place at the experimental site during 1993–1995 was that conducted during the experiment.

Experimental design

Full details of the design of the overall experiment are given in Rowell et al. (1997). Two 13-km-long corridors were established, each containing both a strip to be trawled and a parallel reference strip 300 m to one side (Fig. 1).

The experimental strips were trawled by the C.S.S. *Wilfred Templeman* in three successive years (1993, 1994, and 1995). Pre- and post-trawl benthic surveying and sampling were conducted each year by the C.S.S. *Parizeau*. Each year, samples from the reference strips were collected before trawling the experimental strips. Additional benthic sampling was also conducted in September 1993, 10 weeks after the initial trawling disturbance. With the exception of the *Templeman* in 1993, both ships were equipped with a digital Global Positioning System (dGPS) that has an accuracy of 3–4 m at the experimental site (McKeown and Gordon 1997).

For sampling purposes, each corridor was divided transversely into 260 blocks, each 50 m long, with the blocks spanning both trawled and reference strips. A different selection of blocks (usually 10) was chosen for sampling in each corridor on each cruise using a random procedure (McKeown and Gordon 1997). In 1993, the pre- and post-trawling samples were collected from different selections of blocks, while in 1994 and 1995, they were collected from the same blocks. The grab sampled an area of 0.5 m^2 to a depth of 10–25 cm as close to the centre of a block as possible

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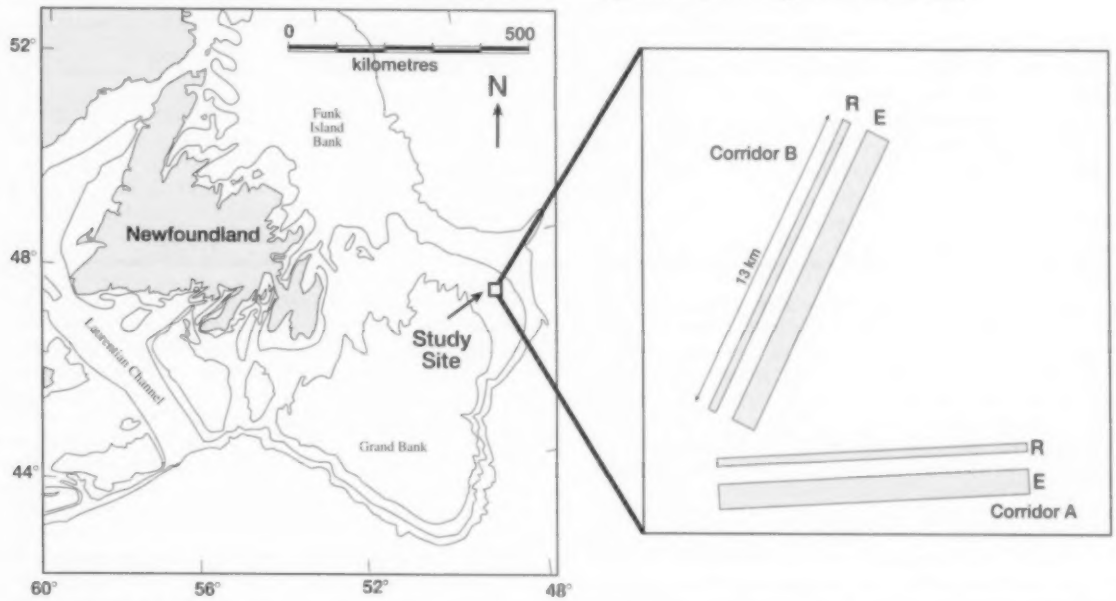
Fig. 1. Location of study site on the Grand Banks off Newfoundland, Canada. Schematic representation of the relative position and orientation of the reference (R) and trawled (E) strips of Corridors A and B within the closed area of the study site. Three corridors were trawled in this experiment (Prena et al. 1999), but only the two sampled with the videograb are shown here.



(Rowell et al. 1997). Two colour video cameras on the grab permitted viewing of the operation on monitors in the ship's bridge and laboratory.

Experimental trawling

The experimental trawling was carried out using an Engel 145 otter trawl, with 1250 kg polyvalent otter boards and fitted with a Scanmar net mensuration system (McCallum and Walsh 1997). As determined by Scanmar, the trawl had wing and door spreads of 20 ± 2 and 60 ± 5 m, respectively. The net was rigged with 46-cm-diameter rockhopper gear and had mesh sizes of 180 mm in the wings and belly with 130 mm in the codend. Similar gear configurations were widely used by the fishing industry on the Grand Bank before the groundfish closures. A 30-mm-square mesh liner was installed in the final 9 m of the 18.5-m-long codend in order to capture organisms that may be damaged but not retained by commercial nets. Trawling speed was approximately 7 $km \cdot h^{-1}$.

Each year, the trawled strips in each corridor (Fig. 1) were towed 12 times (with direction alternating on each set) within a 5-day period in late June – early July (Prena et al. 1999). The time required to trawl the 12 sets in one corridor was generally on the order of 30–35 h. The trawl catch has been described by Prena et al. (1999).

The widths of the disturbance zones created each year by the otter trawling were estimated from the navigation data (Table 1) and in general were confirmed by sidescan sonar information (Schwinghamer et al. 1998). Cumulative trawling disturbance was greatest nearer the centre lines of the trawled strips where the videograb was targeted, but the local intensity of the impact from doors, footgear, net and cables within each strip was necessarily uneven.

Sample processing

On retrieval of the videograb, a 125-mL sample of surface sediment was collected through a sampling port and frozen for organic carbon and nitrogen content analysis. Sediment organic carbon and nitrogen content were determined using a Perkin-Elmer 2400 series II CHN analyzer after an acid treatment to remove carbonates. The videograb contents were then washed through a 1-mm screen and retained material preserved in buffered formalin onboard ship.

Where possible, all organisms were identified to species level. Poor condition of specimens, lack of information about juvenile forms, gaps in taxonomic knowledge of some groups, and lack of expertise necessary for accurate identifications of some types prevented species-level identification for some. Ostracods, bryozoans, and barnacles, although collected, were not analyzed due to their size range and growth form, respectively, creating large variance in the data. Abundance and, for most species, biomass (formalin wet weight, including both mantle cavity liquid and shells for molluscs) were determined for each taxon.

For the brittlestar *Ophiura sarsi* and the sand dollar *Echinarachnius parma*, the proportion of damaged animals in each of three relative size-classes (small, medium, and large) was tabulated. For brittlestars, only damage to the central disc was recorded.

Statistical analysis

For each videograb sample, the percent organic carbon, percent nitrogen, molar carbon to nitrogen ratio, both biomass (B) and abundance (N) of all species combined, number of species, average weight of individuals across species (B/N, using only species for which biomass data was available), Shannon–Wiener diversity index (H', calculated using logarithms to the base 10, i.e., decits per individual), and Pielou's evenness index (J') were calculated. Each of the hypothesis tests described below was performed separately on each of these community indices as well as on the abundances of each individual species or species group and the biomass of the taxa for which weight data had been collected (except when a taxon was absent from a particular subset of the data or where the frequency of occurrence or variance structure precluded statistical analysis). No corrections were made for the volumes of the videograb samples, as most of the fauna were found in the top layers of the sediment, which were always fully sampled.

The analyses of community indices were expected to reveal overall trends in the benthic macrofauna, while the tests of abun-

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Table 1. Numbers of videograb samples collected during the experiment, time interval between the last trawl set and commencement of videograb sampling, and mean width of the disturbance zone created by otter trawling.

		No. of grab samples collected in each of Corridors A and B		Interval between last trawl set and first grab sample (h)		Mean width (SD) of disturbance zone (m)	
Date	Condition	Experimental	Reference	Corridor A	Corridor B	Corridor A	Corridor B
July 1993	Pretrawl	5	5				
	Posttrawl	10		13	12	264 (55)	204 (102)
Sept. 1993	Pretrawl	10	10				
July 1994	Pretrawl	10	10				
	Posttrawl	10		132	32	81 (10)	119 (15)
July 1995	Pretrawl	10	10				
	Posttrawl	10		14	8	121 (9)	112 (16)

dance and biomass disaggregated to the level of individual taxa should illustrate the detailed changes underlying those trends. It was, however, possible that some shifts in community structure would be masked in the indices if various groups of species reacted in opposite ways to trawling impacts, while low statistical power could prevent detection of those changes by the analyses of individual species. Thus, the same suite of hypothesis tests was also applied to the abundance and biomass data when aggregated to the level of taxonomic families. Furthermore, for the polychaetes, the various species were grouped into ecological categories (type: tubicolous, errant, burrowing; feeding mode: filter feeder, carnivore, deposit feeder) following Fauchald and Jumars (1979) and Pocklington (1989), and the same tests were applied to those categories. (Such comprehensive ecological knowledge is not readily available for the macrobenthos of the northwest Atlantic, and it was not possible to make similar ecological groupings of the other major taxa in the same way.) These analyses of families and ecological categories did not reveal any statistically significant patterns that were not also apparent in the single species results, trends in the data for most aggregations being dominated by those of the one or two constituent species that also provided significant results when analyzed individually. Thus, these analyses of larger groupings are not further reported here.

Analysis of variance

The following one-way model I analysis of variance (ANOVA) was used to test Hypothesis 1 that there were no differences between the macrofauna in Corridors A and B immediately prior to the experimental trawling:

$$(1) \qquad \text{Model A: } Y_{il} = \mu + C_i + \varepsilon_{il}$$

where factor C_i represents a corridor effect with levels A and B. The July 1993 samples from the reference and trawled strips (pretrawl samples only) were analyzed (df = 1,18). The samples from within each corridor were not independent, as the same blocks were used across both the trawled and reference strips, increasing the probability of falsely "significant" results (Type I errors). The correlation between the two strips in each corridor was therefore examined for significance using data from the pairs of samples as bivariate data points to determine the severity of this effect (Legendre and Legendre 1998).

A two-way model I ANOVA was used to test Hypothesis 2 that there were no differences in the macrofauna of the reference strips in the four sampling periods:

$$(2) \qquad \text{Model B: } Y_{ijl} = \mu + C_i + D_j + CD_{ij} + \varepsilon_{ijl}$$

where factor D_j is a date effect with four levels (July 1993, September 1993, July 1994, and July 1995) and C is as previously defined (df = 3,62).

The data from each year (1993, 1994, and 1995) were analyzed separately to test Hypothesis 3 that there was no immediate effect of the trawling on the macrofauna in the trawled strips. Since the reference strips were only sampled before each year's trawling, they could not be used as control sites in any short-term study, and so these analyses considered only change in the trawled strips. For the 1993 data, a full-factorial ANOVA was used, as the sample blocks were separately randomised before and after trawling in 1993. However, in 1994 and 1995, two different ANOVA models, one a repeated-measures design and the other a full-factorial ANOVA, were used. Because the pre- and post-trawl samples in 1994 and 1995 were taken from the same blocks (resampled), the appropriate analysis would normally be a repeated-measures ANOVA. That model requires an assumption that there is no error due to differences in sampling location within blocks, and any variance at that level is incorporated into the trawling treatment term. The assumption is unlikely to be accurate in the present case. The repeated-measures ANOVA is therefore expected to err on the side of Type I errors.

The two-way model I full-factorial ANOVA was used to test Hypothesis 3 and applied to data from each of the three years:

$$(3) \qquad \text{Model C: } Y_{ikl} = \mu + C_i + T_k + CT_{ik} + \varepsilon_{ikl}$$

where factor T_k is a treatment effect with two levels (pre- and post-trawl) and C is as previously defined (df = 1,26 for 1993; df = 1,36 for 1994 and 1995). As the blocks were not randomly drawn in 1994 and 1995, this model will underestimate the error term, also erring on the side of Type I errors.

The relative appropriateness of the two-way full-factorial ANOVAs and the repeated-measures model depends on the spatial pattern of variation in the data being analyzed. As the fine-scale distribution of the species was unknown, both types of ANOVA were performed and the results compared. In most cases, both tests produced consistent results, and with few exceptions, only the results of the two-way full-factorial ANOVA are presented in this paper.

A three-way model I ANOVA was applied to data from the reference and pretrawl samples from July 1993, 1994, and 1995 to test Hypothesis 4 that there was no long-term effect of the trawling on the macrobenthos in the trawled strips:

$$(4) \qquad \text{Model D: } Y_{ijkl} = \mu + C_i + D_j + T_k + CT_{ik} + DT_{jk} + CD_{ij} + CDT_{ijk} + \varepsilon_{ijkl}$$

where factor D_j is a date effect with three levels (July 1993, July 1994, and July 1995) and the other factors are as previously defined (df = 2,88). In this model, a significant interaction term, DT_{jk} or CDT_{ijk}, would represent a long-term cumulative effect of trawling.

This analysis was pseudoreplicated because the videograb samples, instead of the corridors, were used as replicates. This also will tend to create Type I errors. Analyzing the data with the two corridors as replicates would have so reduced the degrees of freedom as to eliminate any prospect of detecting significance, thus

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incurring Type II errors. The model was applied, despite its obvious error, since its nonsignificant results are valid, although the few significant ones must be interpreted with caution.

In cases of these ANOVAs, results were deemed significant against a standard of $\alpha = 0.01$. With the very large number of tests performed, application of the Bonferroni procedure would require such a low level of α that the tests would have had extremely low power and could only have yielded Type II errors. Conversely, using the conventional $\alpha = 0.05$ could have resulted in some hundreds of Type I errors. Consequently, we chose a level of α that will have produced a moderate number of falsely significant results, which must be kept in mind when interpreting the analyses.

Before conducting each of these tests, the distributions of the data subsets (assumed indicative of those of the error terms) were examined through normal and detrended normal *Q–Q* plots of residuals. The sample means were plotted against the sample variances, and homogeneity of variances was tested with Levene's test. Where the distributions were not normal and (or) the variances were unequal, data were logarithm ($\ln(x + 1)$) transformed and these assumptions were reassessed. If the error term was normally distributed but the variances were unequal, a Welch one-way ANOVA was performed to test the equality of means allowing unequal variances. The Welch ANOVA (equivalent to an adjusted Student's *t* test when there are two groups) only tested the significance of one main effect. Tukey's honestly significant difference (HSD) test was used as a post hoc analysis to determine which pairs of sample means were significantly different. The power and least significant number of all tests were calculated but only reported if relevant.

Multidimensional scaling

A dissimilarity matrix was calculated using the Bray–Curtis metric and the full matrix of species abundances ($n = 246$) from each of the videograb samples ($n = 200$). The data were $\log_{10}(x + 1)$ transformed before calculation of the dissimilarities. The relationships among the samples were then ordinated by nonmetric multidimensional scaling (MDS). Euclidean distance was used as the scaling model. An iterative algorithm designed to minimize the s-stress goodness-of-fit criterion was used (Young's s-stress formula 1), with iterations proceeding until the improvement in s-stress was less than 0.001 from one iteration to the next or when s-stress reached 0.005 or less. Kruskal's stress index (formula 1) and R^2 were also calculated. A cascading number of dimensions were calculated independently. The two-dimensional configuration was displayed in the figures wherever possible to facilitate viewing, although this number of dimensions was not always the best representation of the data.

Individual videograb samples were identified in the MDS plots by corridor, treatment, and date to explore possible explanations of observed biological structuring. Ordinations, which showed evidence of structuring, were further examined to determine the correlation of individual species abundances with the MDS configuration. The significance of these correlations was adjusted ($\alpha = 0.01$) to account for the number of tests performed.

Results

Sediment organic carbon, nitrogen, and carbon to nitrogen ratio

The percentages of organic carbon and nitrogen were low and the molar carbon to nitrogen ratio was 7.67 ± 1.73, which is characteristic of fresh organic matter. These values are typical of the moderately to well-sorted fine- to medium-grained sands. ANOVAs of these chemical data in general gave nonsignificant results. However, after trawling in 1995, the percentage of organic carbon decreased significantly from an average of 0.09% to 0.07%, while the percentage of nitrogen decreased significantly from an average of 0.015% to 0.010% (ANOVA Model C: $F = 11.95$, $P = 0.001$ and $F = 10.15$, $P = 0.003$, respectively).

General description of the macrobenthic community

The undisturbed macrofaunal community at the study site was relatively homogenous and typical of clean, offshore sandy seabeds in the northwest Atlantic. Two hundred and forty-six invertebrate species or species groups, belonging to 120 families, were recorded from the 200 videograb samples. Sixty-seven species of polychaetes, 51 of crustaceans, 23 of bivalves, 52 of gastropods, and 12 of echinoderms were fully identified. Twenty-seven taxa were found in more than 90% of the samples, while 122 occurred in less than 10%. The total number of species and species groups per sample ranged from 31 to 87, with an average of 67.8, and declined over the 2 years of the experiment (Fig. 2) due primarily to the loss of 31 crustacean (mostly amphipods) and 23 molluscan species. A number of species showed temporal variability over the study period.

Nineteen taxa collectively accounted for 81–87% of the numbers of animals in each sample. The principal species included the polychaetes *Prionospio steenstrupi*, *Chaetozone setosa*, *Spio filicornis*, and *Nothria conchylega* as well as the amphipod *Priscillina armata*, the bivalve mollusc *Macoma calcarea*, and the sand dollar. *Macoma calcarea* and *Prionospio steenstrupi* usually had the greatest abundance. The total abundance per videograb sample (0.5 m^2) ranged between 252 and 2291 individuals, with an overall average of 1064, and declined sharply through the course of the study (Fig. 2).

The biomass was dominated by propeller clams (*Cyrtodaria siliqua*) and sand dollars, two species that are commonly associated. The brittlestar *Ophiura sarsi*, the bivalve *Macoma calcarea*, and the sea urchin *Strongylocentrotus pallidus* also contributed substantially to biomass. All are important dietary constituents of demersal fish (e.g., Methven 1999) and were also present in high abundance. Twenty-three species accounted for approximately 95% of the total biomass. In general, biomass was more stable with year and treatment than was abundance. The total biomass per videograb sample (0.5 m^2), summed across all species, ranged from 149.24 to 1102.88 g with an overall average of 513.61 g. The average weight of an organism (*B*/*N*) in a grab sample ranged from 9.3 to 321 g with an overall average weight of 69.4 g. This ratio was calculated for taxa where both biomass and abundance data were available and not through a simple division of total recorded biomass by total recorded number per sample. Both total biomass and *B*/*N*, while variable, showed no particular pattern or change through the study (Fig. 2).

The Shannon–Wiener diversity index (H') for each sample varied between 0.96 and 1.45, while Pielou's measure of evenness (J') varied from 0.52 to 0.76 among the 200 samples (Fig. 2). Through the course of the experiment, J' and H' of the community varied (Fig. 2), creating a parallel dome-shaped pattern.

Comparison of Corridors A and B before trawling

The ANOVAs designed to test the null hypothesis of no differences between the corridors before the experiment (Model A) produced few significant results (Table 2). Of the

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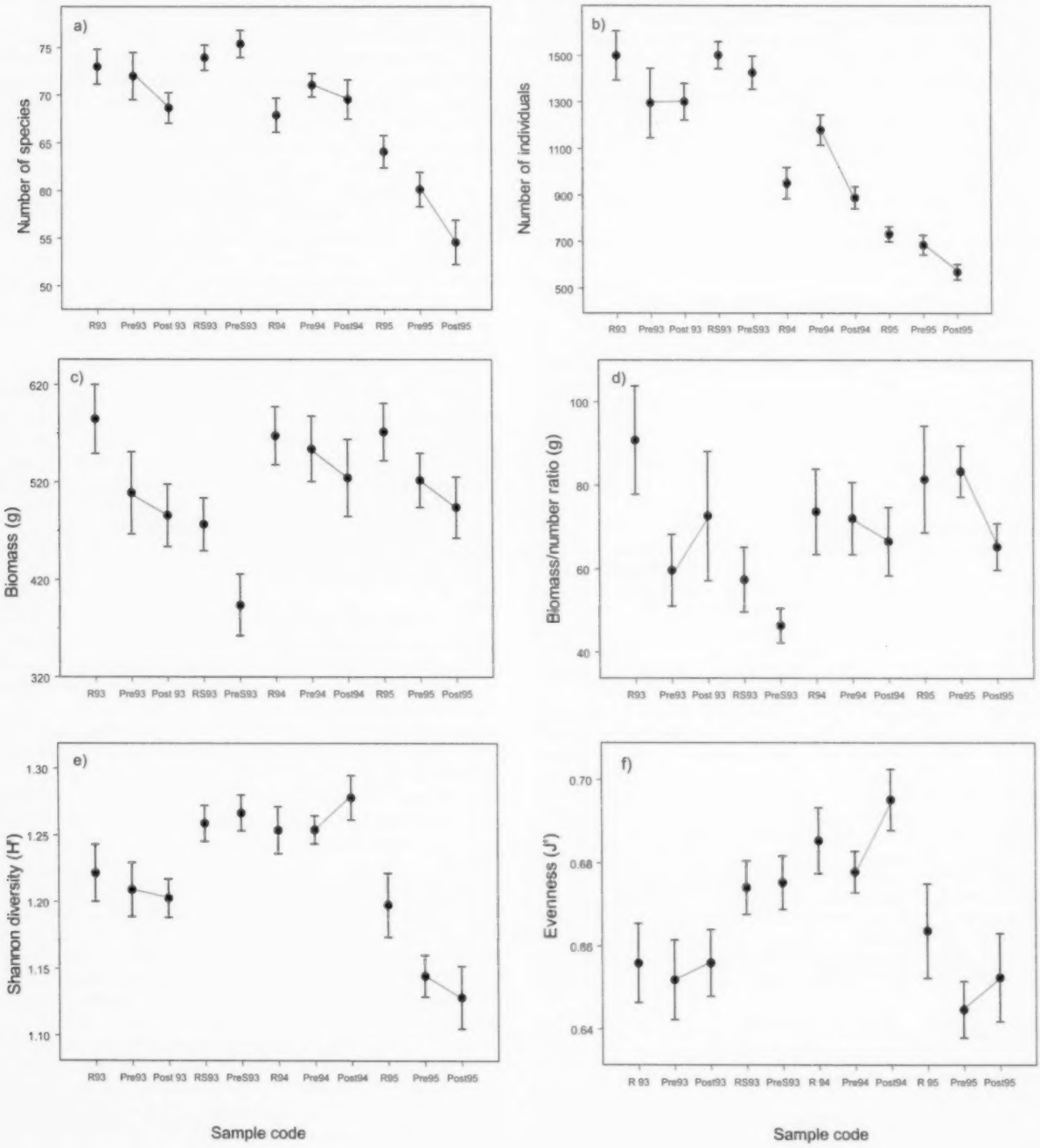
Fig. 2. Means and standard error for (*a*) total number of species, (*b*) total number of individuals, (*c*) total biomass, (*d*) biomass/number ratio (average weight of an organism in the sample), (*e*) Shannon diversity, and (*f*) Pielou's evenness per grab sample (0.5 m^2) for each of 11 sample units (R, reference strip; Pre, experimental strip before trawling; Post, experimental strip after trawling; S, September; 93, 1993; 94, 1994; 95, 1995). Pre- and post-trawling samples each year are connected by a line.



170 taxa individually analyzed, only seven showed significant differences in abundance between the corridors, while six of 161 showed significant differences in biomass. With the exception of the bivalve *Yoldia myalis*, all of the species that showed significant differences in biomass were also found to show significant differences in abundance.

Correlation between the trawled and reference strips, by sample block, ranged from 0.0 to 0.50 in Corridor A and

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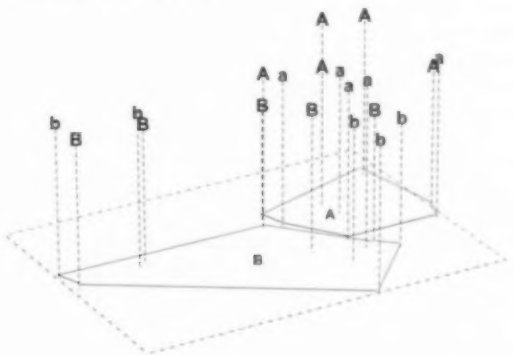
Table 2. Summary of those community indices and individual species abundance and biomass that showed significant between-corridor differences before trawling commenced in 1993 in Model A ANOVA.

Index/species	Mean		r^2		F	Probability of F
	Corridor A	Corridor B	Corridor A	Corridor B		
No. of species per sample	68.50	76.50	0.07	0.00	10.569	0.004
Abundance (no.·0.5 m^{-2})						
Thyasira sp. A (M)	10.68	3.89	0.18	0.18	24.580	0.000
Galathowenia oculata (P)	2.00	3.92	0.00	0.02	16.309	0.001
Nereimyra punctata (P)	3.92	4.32	0.50	0.29	26.071	0.000
Monoculopsis longicornis (C)	2.00	3.10	0.00	0.26	16.848	0.001
Oediceros borealis (C)	2.42	5.44	0.03	0.26	13.043	0.002
Eudorellopsis deformis (C)	3.69	11.18	0.02	0.17	16.123	0.001
Priscillina armata (C)	31.88	150.90	0.43	0.62	12.159	0.003
Biomass (mg·0.5 m^{-2})						
Thyasira sp. A (M)	425.11	22.12	0.00	0.21	20.881	0.000
Yoldia myalis (M)	12.94	1013.32	0.29	0.83	13.207	0.002
Nereimyra punctata (P)	41.45	12.94	0.04	0.19	16.034	0.001
Monoculopsis longicornis (C)	2.00	3.48	0.00	0.86	16.171	0.001
Oediceros borealis (C)	2.65	4.06	0.06	0.11	9.517	0.006
Eudorellopsis deformis (C)	3.48	9.58	0.53	0.07	9.375	0.007

Note: The untransformed geometric mean values (calculated from the $\ln(x + 1)$-transformed mean values used in ANOVA) across samples and the correlation (r^2) between trawled and reference strips within corridors are indicated. $r^2 < 0.92$ are nonsignificant at $\alpha = 0.01$. M, Mollusca; P, Polychaeta; C, Crustacea.

Fig. 3. Three-dimensional MDS configuration of the 1993 predisturbance grab samples based on Bray–Curtis dissimilarity from $\log_{10}$-transformed abundance of 170 species. Corridor is indicated as A or B. Experimental strips are represented by uppercase letters and reference strips by lowercase letters. The area on the X–Y plane occupied by each corridor (both reference and experimental strips) is mapped.



from 0.02 to 0.62 in Corridor B, indicating substantial, although nonsignificant, deviations from the assumption of independence in some cases. The r^2 values were generally somewhat higher for biomass: 0–0.53 in Corridor A and 0.07–0.86 in Corridor B (Table 2). Among the community indices, only the number of species per grab sample showed a significant difference between the corridors (more in Corridor B); however, the power of the tests was low (0.25–0.40). These analyses show that while the macrofaunal communities in the two corridors were detectably different before the trawling, those differences were minimal.

The predisturbance relationships between the samples from both reference and trawled strips are displayed in an MDS plot (Fig. 3). The abundances of 170 species were used to derive the Bray–Curtis metric. The ordination had a stress level of 0.075 and an R^2 of 0.96, indicating an excellent representation of the data. The data separate into groups that are consistent with corridor affinity, supporting the ANOVA. However, the greatest separation of the samples is seen between four Corridor B samples and the rest, while the separation between Corridor A and remaining Corridor B samples is less prominent (Fig. 3).

Temporal changes in the reference strips

The tests of the null hypothesis of no differences in the reference strips over time (Model B) produced a considerable number of significant results (Table 3). There was a clear trend of decreasing total abundance in the reference strips from September 1993 onwards (Fig. 2). It dropped approximately 50% between July 1993 and July 1995. Of 209 taxa tested individually, 43 species showed a significant date effect (Table 3). Seventeen of them showed the same pattern in post hoc analysis: a decline in abundance between September 1993 and July 1994. The molluscan family Trochidae and the carnivorous polychaete *Phyllodoce groenlandica* were the only taxa to increase significantly in abundance from 1993 to 1995 in the reference strips.

In ANOVAs of the biomass of 191 individual taxa, 35 showed significant date effects (Table 3) and five showed a significant date × corridor interaction. However, there was no significant change in total biomass per sample. Post hoc analysis showed that 10 of the 21 polychaete species showed the same temporal pattern as was seen in the abundance data, namely a drop in biomass between September 1993 and July 1994 followed by no further significant change. Only two taxa, an unidentified Gastropoda and the Trochidae, increased significantly in biomass in the reference strips during the experiment.

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Table 3. Species and community indices that showed significant ($P < 0.01$) changes over time in abundance and (or) biomass in reference samples in Model B ANOVA, with the significantly different sample periods indicated.

Species	Index or abundance	Biomass
Total abundance per grab	(J93, S93) > J94 > J95	
Total no. of species per grab	(J93, S93) > J94 > J95	
Polychaeta juvenile	(J93, S93) < J94 > J95	ns
Capitella capitata (P)	(J93, S93) > (J94, J95)	ns
Mediomastus ambiseta (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Nothria conchylega (P)*	(J93, S93, J94) > J95	(J93, S93, J94) > J95
Ophelina breviata (P)	J93 < S93 > (J94, J95)	J93 < S93 > (J94, J95)
Aricidea catherinae (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Aricidea wassi (P)	J93 < S93 > (J94, J95)	J93 < S93 > (J94, J95)
Nereimyra punctata (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Pholoe minuta (P)	(J93, S93) > (J94, J95)	J93 > S93 > J94 > J95
Polynoidae juvenile (P)	J93 < S93 > (J94, J95)	(J93, S93) > (J94, J95)
Apistobranchus typicus (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Chaetozone setosa (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Polydora concharum (P)	ns	J93 > S93 > J94 > J95
Polydora socialis (P)	(J93, S93) > J94 > J95	J93 > S93 > J94 > J95
Prionospio steenstrupi (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Ampharete finmarchica (P)	(J93, S93, J94) > J95	ns
Pectinaria hyperborea (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Polycirrus medusa (P)	(J93, S93) > (J94, J95)	J93 > (S93, J94, J95)
Eteone longa (P)	(J93, S93) > (J94, J95)	ns
Dorvilleidae undetermined spp. (P) (a)†	(J93, S93) > (J94, J95)	J93 > (S93, J94, J95)
Spio filicornis (P)	(J93, S93, J94) > J95	(J93, S93) > (J94, J95)
Phyllodoce groenlandica (P)	(J93, S93, J94) < J95	ns
Gattyana cirrosa (P)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Chaetozone sp. (P)	(J93, S93, J94) > J95	(J93, S93, J94) > J95
Lysippe labiata (P)	(J93, S93, J94) > J95	ns
Jasmineira sp. (P)	ns	J93 < S93 > (J94, J95)
Leaena ebranchiata (P)	ns	J93 > (S93, J94, J95)
Guernea nordenskioldi (C)	(J93, S93) > (J94, J95)	(J93, S93) > (J94, J95)
Protomedeia fasciata (C) (b)†	(J93, S93) > (J94, J95)	J93 > S93 > J94 > J95
Harpinia plumosa (C)	(J93, S93) > J94 > J95	(J93, S93) > (J94, J95)
Phoxocephalus holbolli (C)	(J93, S93) > J94 > J95	(J93, S93) > (J94, J95)
Eudorellopsis deformis (C)	(J93, S93, J94) > J95	(J93, S93, J94) > J95
Leptognathia sp. (C)	J93 > (S93, J94, J95)	J93 < S93 > J94 > J95
Ampelisca macrocephala (C)	J93 < S93 > (J94, J95)	J93 < S93 > (J94, J95)
Metopa sp. 3 (borealis type) (C)	J93 > (S93, J94, J95)	(J93, S93) > (J94, J95)
Byblis gaimardi (C)	(J93, S93) > (J94, J95)	(J93, S93) > J94 > J95
Diastylis rathkei (C)	J93 > (S93, J94, J95)	ns
Stenula peltata (C)	ns	(J93, S93) < J94 > J95
Oediceros borealis (C)	(J93, S93) > (J94, J95)	ns
Leucon nasicoides (C) (a, b)†	J93 > S93 > (J94, J95)	J93 > S93 > (J94, J95)
Echinarachnius parma (E)	(J93, S93, J94) > J95	ns
Clinocardium ciliatum (M)	J93 < S93 > (J94 > J95)	ns
Cylichna alba (M)	(J93, S93, J94) > J95	ns
Trochidae spp. (M)	(J93, S93) < (J94, J95)	(J93, S93) < J94 < J95
Curitotoma trevelliana (M)	J93 < (S93, J94) > J95	(J93, S93) < J94 > J95
Gastropoda spp. (M)	ns	(J93, S93) < (J94, J95)
Gersemia sp. (Cn)	(J93, S93, J94) > J95	ns
Nemertea undetermined spp.	(J93, S93) > J94 > J95	ns

Note: All lower-level interactions were nonsignificant for these taxa unless otherwise indicated. Sampling period denoted as J for July and S for September followed by the two-digit year. P, Polychaeta; C, Crustacea; E, Echinodermata; M, Mollusca; Cn, Cnidaria. ns, nonsignificant.

*From Welch ANOVA due to unequal variances.

†Significant interaction term in the analysis of abundance (a) and (or) biomass (b).

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Table 4. Species and community indices that showed significant ($P < 0.01$) changes before and after trawling in Model C ANOVA of the abundance and (or) biomass for each year.

		Mean abundance (no.·0.5 m^{-2})			Mean biomass (mg·0.5 m^{-2})		
Year	Species/index	Pretrawl	Posttrawl	% change	Pretrawl	Posttrawl	% change
1993	*Protomedeia fasciata* (C)	3.82	2.50	–34	ns	ns	ns
	Monoculodes intermedius (C)	ns	ns	ns	3.26	2.14	–34
1994	Total abundance per sample	1148.97	867.46	–25			
	Capitella capitata (P)	4.66	2.77	–40	ns	ns	ns
	Mediomastus ambiseta (P)	9.15	4.67	–49	16.97	8.61	–49
	Eteone longa (P)	24.20	12.30	–49	ns	ns	ns
	Apistobranchus typicus (P)	6.88	3.68	–47	13.38	5.60	–58
	Chaetozone setosa (P)	99.30	44.64	–55	253.65	124.97	–51
	Polydora socialis (P)	12.40	4.94	–60	31.88	7.77	–76
	Prionospio steenstrupi (P)	174.99	58.00	–67	399.62	119.39	–70
	Spio filicornis (P)	89.23	36.80	–59	284.44	98.91	–65
	Ophelina breviata (P)	3.67	2.47	–33	4.21	2.69	–36
	Aricidea catherinae (P)	14.74	7.07	–52	52.73	19.12	–64
	Aricidea wassi (P)	4.45	2.64	–41	3.75	2.32	–38
	Polynoidae juvenile (P)	5.34	2.75	–48	3.20	2.23	–30
	Oligochaeta undetermined spp. (O)	7.04	3.32	–53	ns	ns	ns
	Yoldia myalis (M)	3.11	4.31	39	ns	ns	ns
	Echinarachnius parma (E)	ns	ns	ns	155 283.30	98 126.25	–37
	Protomedeia grandimana (C)	3.00	4.82	61	5.48	13.35	144
1995	*Ophelia limacina* (P)*	2.21	2.78	26	ns	ns	ns
	Polynoidae juvenile (P) (b)*	4.51	3.92	–13	2.09	2.01	–4

Note: The untransformed geometric mean abundance (calculated from the $\ln(x + 1)$-transformed means used in ANOVA) before and after trawling and percent change are given. Interactions were nonsignificant. ns, nonsignificant. C, Crustacea; P, Polychaeta; O, Oligochaeta; M, Mollusca; E, Echinodermata.

*Repeated-measures ANOVA; "b" indicates repeated-measures analysis of biomass only.

The number of species per sample also decreased significantly during the experiment, with the biggest drop occurring between July 1994 and July 1995 (Fig. 2), the decline being on the order of four species per sample. None of the other community indices showed significant temporal change.

The very large number of significant results, plus the sharp drop in the number of species and total abundance (Fig. 2), leaves little doubt that the macrofaunal communities in the reference strips experienced considerable change during the 3 years of the experiment, despite the possibility of Type I errors. While the reference strips in the two corridors had rather different benthic communities (Fig. 3), the temporal changes affected them both in a parallel manner. Only five species showed significant interactions of date and corridor, which is about the number expected by random chance alone.

The relationships between the faunal compositions in samples from the reference strips collected in the three July sampling periods, identified by corridor and date, are displayed in Fig. 4 in a two-dimensional MDS plot. The abundances of 198 taxa were used to derive the Bray–Curtis metric. The MDS had a stress level of 0.224 and an R^2 of 0.78, indicating a reasonable representation of the data. Several of the post hoc analyses of individual taxa indicated that the compositions in the 1993 samples were different from those taken in 1994 and 1995 (Table 3), and this difference is apparent in the MDS plot. When identified by corridor (not shown) the 1993 samples were discrete from the 1994 and 1995 samples in both Corridors A and B.

The taxa that were most highly correlated with the MDS axes were a mixture of small polychaetes (e.g., *Pholoe minuta*, *Nothria conchylega*, *Aricidea catherinae*, and *Prionospio steenstrupi*), crustaceans (e.g., *Harpinia plumosa* and *Sympleustes pulchellus*), the soft coral *Gersemia* sp., molluscs, and others. Twelve taxa were significantly correlated with the first MDS dimension and 52 with the second. Both dominant and rarer species influenced the position of the samples. In combination with those from the Model B ANOVAs, these results show that the macrofaunal community in the study area is naturally dynamic and exhibits marked changes irrespective of trawling activity.

Immediate effects of trawling

In 1993, the mean total abundance per sample was very similar before and after trawling (Fig. 2). However, in 1994, it was substantially (25%) lower after trawling. The Model C ANOVA confirmed that the decrease in mean total abundance in 1994 was statistically significant (Table 4). In 1995, the mean total abundance was lower after trawling (Fig. 2), but this difference was not significant.

The analyses also identified some significant effects of trawling on the abundances of particular taxa (Table 4), with most of those occurring in 1994. In that year, 15 taxa showed a significant effect, with 13 of those, mostly polychaetes, showing a decrease in abundance after trawling that ranged from 33 to 67%. However, two species, the bivalve *Yoldia myalis* and the amphipod *Protomedeia grandimana*, increased 39 and 61% respectively.

Each year, the mean biomass per sample was lower after trawling (Fig. 2), but the Model C ANOVA showed that this

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Fig. 4. Two-dimensional MDS configuration of the July grab samples from the reference strips of Corridors A and B based on Bray–Curtis dissimilarity from $\log_{10}$-transformed abundance of 198 species. Samples represented by circles denote 1993 collections, triangles denote 1994 collections, and squares denote 1995 collections. Sample boundaries by year are indicated.

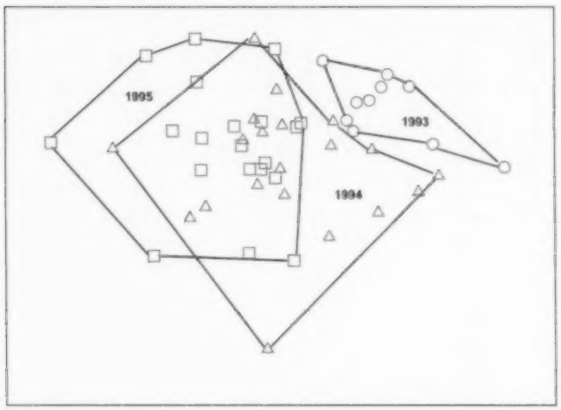
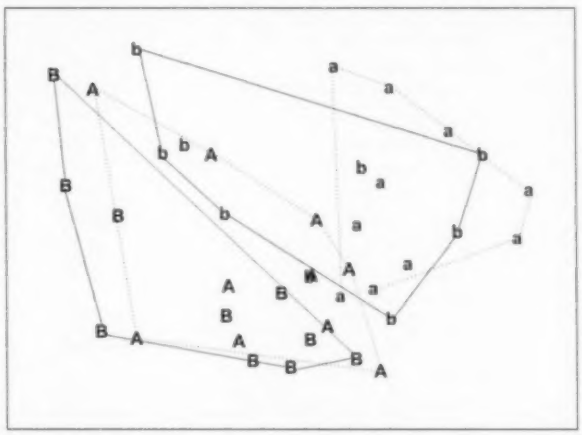


Fig. 5. Two-dimensional MDS configuration of the 1994 grab samples from the experimental strips of Corridors A and B based on Bray–Curtis dissimilarity from $\log_{10}$-transformed abundance of 181 species. Samples represented by uppercase letters denote the pretrawl samples and by lowercase letters represent the samples taken after trawling. The areas bounded by the samples from each corridor before and after trawling are denoted by a dotted line for Corridor A and a solid line for Corridor B.



difference was not statistically significant at any time. In 1994, 11 taxa, mostly polychaetes, were negatively impacted (30–76% decrease in biomass), while one species, the amphipod *Protomedeia grandimana*, increased in biomass by 144% (Table 4). With the exception of the sand dollar, the species that showed an immediate effect of trawling on biomass in 1994 also showed an immediate effect on abundance in that year (Table 4).

All species for which the factorial ANOVA models produced significant results also had significant results with the repeated-measures model. Two additional taxa produced significant results with the repeated-measures model only (Table 4).

The proportions of damaged sand dollars and brittlestars in the videograb samples were not significantly different in the pre- and post-trawling samples in any of the three years, suggesting that the damage observed was due to sampling. The overall mean number of damaged sand dollar specimens was 13%, but on average, 4% of the small, 16% of the medium, and 24% of the large animals were damaged. Brittlestars sustained less damage, with an overall average 3% affected. The small brittlestars were not damaged at all, while more of those in the medium size-class (7%) sustained damage than those in the large size-class (3%).

None of the other community indices in any of the years showed a significant immediate effect of trawling, and MDS analysis did not indicate any changes in community structure that could be explained by trawl state, other than in 1994 (Fig. 5). The two-dimensional configuration of the samples from the trawled strips in that year was based on a matrix of 181 species. It had a stress level of 0.244 and an R^2 of 0.70, indicating a reasonable representation of the data. The samples separated into two clusters that were consistent with trawling disturbance (the separation between pre- and post-trawling samples is complete in Corridor B data and with a small overlap in Corridor A data). This suggests that the trawling in 1994 caused sufficient disturbance to alter the composition of the macrofaunal benthic community in the short term. The small polychaetes and *Protomedeia grandimana*, which showed a significant effect in the ANOVAs (Table 4), were most highly correlated with the MDS positions of the samples. In addition, a number of molluscs (e.g., *Thyasira* sp. A, *Cyrtodaria siliqua*, and *Curitotoma laurenciana*) were highly correlated with the sample positions in the MDS. Rarer species were correlated with both axes, indicating that the MDS was not driven by the numerically dominant species.

Long-term effects of trawling

The ANOVA designed to test the hypothesis that there was no long-term effect of trawling on the macrobenthos (Model D) produced very few significant interactions indicative of long-term trawling effects (Table 5). Differences between the corridors and sampling dates were found, both in some community indices and in individual taxa, but they do not add to the results of other analyses already described.

Total abundance per videograb sample did show a significant date × treatment interaction, and similar significant interactions were found in eight individual species of polychaetes (Table 5). For each of the eight and for total abundance, the mean abundance in the reference strips fell from 1993 to 1994, remaining low in 1995, while the equivalent value for the trawled strips remained high from 1993 to 1994 but fell by 1995.

The biomass of individual species showed a similar pattern, with three displaying significant interactions. Each of those three also showed a significant response in their abundance (Table 5). Once again, the pattern of a decrease in the reference strips by 1994, with a similar decrease in the trawled strips a year later, was repeated. This distinctive pattern is not consistent with the annual application of trawling as a causative factor.

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Table 5. Polychaete species and index that showed significant ($P < 0.01$) date × treatment interactions in Model D ANOVA of the abundance and (or) biomass from the July pretrawl samples.

Index/species	*F*	Probability of *F*
Total abundance per sample	5.999	0.004
Abundance		
Capitella capitata	5.378	0.006
Mediomastus ambiseta	6.179	0.003
Eteone longa	7.218	0.001
Apistobranchus typicus	7.275	0.001
Chaetozone setosa	16.608	0.000
Polydora socialis	8.153	0.001
Prionospio steenstrupi	11.053	0.000
Spio filicornis	11.161	0.000
Biomass		
Chaetozone setosa	10.735	0.000
Prionospio steenstrupi	9.632	0.000
Spio filicornis	9.572	0.000

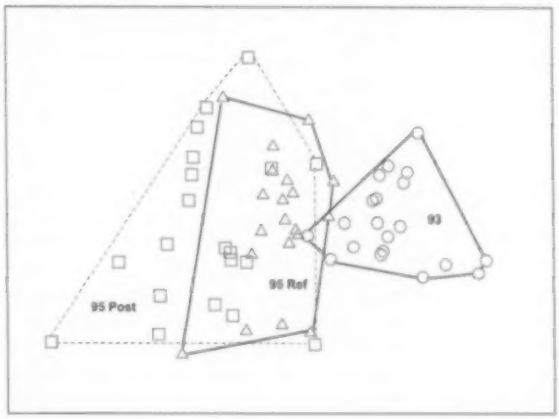
Note: All corridor × date × treatment interactions were nonsignificant.

Given that an immediate effect of trawling was only observed in 1994, "recovery" could only be expected for those few species that responded to the disturbance in that year and also for total abundance, which responded similarly. Since no consistent long-term effect could be attributed to the trawling disturbance, it would appear that "recovery" for these few species required less than 1 year. Within this timeframe, the actual recovery period is unknown.

The overall effect of trawling on the benthic community was examined by an MDS analysis (Fig. 6). A Bray–Curtis dissimilarity matrix of samples from the 1993 and 1995 reference and trawled strips of both Corridors A and B, using data on 196 species, was the basis of the MDS. For 1993, only the pretrawl samples were used, but for the trawled strips in 1995, only the post-trawl data were included. The MDS ordination had a stress level of 0.189 and an R^2 of 0.85, indicating a good representation of the data. Quite clearly, the largest driving force over the 3-year period was time, as the 1993 and 1995 data are nearly separated on the first dimension (Fig. 6). No differences between Corridors A and B were apparent in this ordination (not shown). The apparent effect of trawling moved the community in the same direction on the ordination axes as the naturally occurring trend (Fig. 6). That is, the species impacted by natural events are also those that are impacted by trawling activity, and the direction of that impact is also the same. The second axis of this MDS plot reflects variability in the benthos, with some samples from each strip having high values and others low values, particularly in 1995.

A partial list of species and species groups whose abundances were significantly correlated ($\alpha = 0.01$, $r = 0.38$) with the axes of this MDS ordination is presented in Table 6. Eleven taxa (eight species of polychaetes, two amphipods, and the brittlestar *Ophiura sarsi*) had 70% or more of their variance explained by the first dimension of the MDS. All of these were positively correlated with this axis and thus declined during the course of this experiment. Many other species had a significant correlation with the first axis, and almost all of them decreased in abundance over time. Fewer species were significantly correlated with the second dimension. Only two species, the sea urchin *Strongylocentrotus pallidus* and the polychaete *Harmothoe imbricata*, had more than 55% of their variance explained by the second dimension.

Fig. 6. Two-dimensional MDS configuration of the 1993 (reference and experimental pretrawl) and 1995 (reference and experimental posttrawl) grab samples based on Bray–Curtis dissimilarity from $\log_{10}$-transformed abundance of 196 species. Samples represented by circles denote 1993 collections, triangles denote 1995 reference collections, and squares denote 1995 experimental posttrawl collections. Areas bounded by each sample unit are denoted.



Unaffected species

Graphical examination, by sample date and treatment, of the transformed mean biomass of the taxa that were present in more than 95% of the 200 videograb samples identified 10 that appeared unaffected by both trawling and natural disturbance. These near-ubiquitous species showed little variation around their mean abundances, suggesting genuine stability rather than a lack of detectable change. Included in this list were six polychaetes, four of which are surface deposit feeders producing tubes on or near the surface (*Ampharete finmarchica*, *Lysippe labiata*, *Laphania boecki*, and the Maldanid group) that would appear vulnerable to disturbance. The molluscs *Astarte borealis*, *Cyrtodaria siliqua*, and *Macoma calcarea* and the amphipod *Priscillina armata* completed the group.

Discussion

This experiment, the first to examine the long-term effects of repeated otter trawling on the infauna of a deepwater sandy site, found a natural long-term decline in the macrofaunal assemblage of the untrawled reference strips as the most prominent feature of the data. There was a sharp fall in both the number of species present and their total abundance as well as in the biomass and abundance of 43 of the 209 species analyzed. Many of those declined between September 1993 and June 1994 and did not subsequently recover. This change coincided with a detectable decrease of the fine fraction (<500 μm) of the sediments in the study area (Schwinghamer et al. 1998). Shorter-lived polychaetes, amphipods, and cumaceans were particularly affected, although

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Table 6. Species that showed significant ($P < 0.01$) correlation and $r \geq 0.50$ between individual abundance in the July 1993 pretrawl and 1995 posttrawl and reference samples and MDS scores calculated from the same data ($\alpha = 0.01$, $r = 0.38$).

Dimension 1		Dimension 2	
Species	r	Taxon	r
Chaetozone setosa (P)	0.92	*Strongylocentrotus pallidus* (E)	0.64
Prionospio steenstrupi (P)	0.92	*Harmothoe imbricata* (P)	0.57
Mediomastus ambiseta (P)	0.80	Polychaeta juvenile	0.51
Harpinia plumosa (C)	0.79	*Clinocardium ciliatum* (M)	0.50
Aricidea catherinae (P)	0.74		
Pholoe minuta (P)	0.73		
Apistobranchus typicus (P)	0.72		
Pectinaria hyperborea (P)	0.72		
Phoxocephalus holbolli (C)	0.71		
Ophiura sarsi (E)	0.73		
Nothria conchylega (P)	0.70		
Nereimyra punctata (P)	0.69		
Eudorellopsis deformis (C)	0.68		
Nemertea undetermined spp.	0.66		
Polycirrus medusa (P)	0.66		
Polydora socialis (P)	0.65		
Ampharete finmarchica (P)	0.64		
Spio filicornis (P)	0.62		
Aricidea wassi (P)	0.61		
Protomedeia fasciata (P)	0.59		
Monoculodes intermedius (C)	0.58		
Capitella capitata (P)	0.57		
Echinarachnius parma (E)	0.57		
Gersemia sp. (Cn)	0.56		
Oediceros borealis (C)	0.55		
Leptognatha sp. (C)	0.53		

Note: P, Polychaeta; C, Crustacea; E, Echinodermata; Cn, Cnidaria; M, Mollusca

the brittlestar *Ophiura sarsi* and the soft coral *Gersemia* sp. also declined.

Beyond this natural trend, there were no additional interannual changes that could be attributed to the impacts of trawling. Such nonsignificant, trawling-related trends as were revealed by MDS paralleled the natural trend seen in the reference samples: trawling disturbance appeared to affect the same species in the community in similar proportions as the natural disturbance. There was no sign of a distinctive trawling disturbance "fingerprint."

There were significant immediate declines associated with the trawling activities in the abundance and biomass of a number of species, most of them polychaetes, but (with few exceptions) only in 1994. That year, sand dollar biomass also fell by more than a third between the pre- and posttrawling periods. Dynamic changes in polychaete populations in response to disturbance are well documented. Their rapid recoveries are attributed to the opportunistic nature of the mobile, scavenging species and the ability of surface tube dwellers to reproduce and in some cases regenerate rapidly. *Capitella capitata*, which was among the species impacted by trawling, has been shown to recover from experimental disturbance in 2 days (Bonsdorff and Pearson 1997). Recovery from these immediate declines seemed to be complete within a year — or at least the differences between the trawled and naturally varying reference areas were reduced to the point of being undetectable by this experiment within that time frame. Only the amphipod *Protomedeia grandimana* showed a major biomass increase across a single trawling event, and that only in 1994.

One feature of our results is that immediate impacts of trawling were only clearly observed in 1994. A chance disruption of the data, such as more of the 1994 samples being taken from the highly disturbed track of an otter board, is unlikely to explain this anomaly, since there was no particular sign of heterogeneity of the variances among the three years. More probably, the greater concentration of the experimental trawling along the centres of the strips in 1994, where the samples were collected, led to more intense impacts on the points sampled. The delay between the end of trawling and the videograb sampling (substantially greater in 1994 than in the other years) may also have allowed more time for mobile scavengers to feed on any infauna exposed on the surface after trawling or for dead or damaged animals to be transported from the site. A rapid influx of snow crabs into the trawled strips was observed (Prena et al. 1999).

The field sampling for this study did not fully meet with the experimental design; however, all of the departures from appropriate design will have increased the probability of Type I errors above the nominal value of $\alpha = 0.01$. Likewise, the very large number of tests performed should have generated multiple Type I errors when interpreted against such a

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Table 7. Polychaete species that showed significant declines through the course of the experiment in abundance and (or) biomass due to the combination of trawling disturbance and natural processes.

Phylum	Order	Family	Species	Guild
Annelida	Capitellida	Capitellidae	*Capitella capitata*	SMX
			Mediomastus ambiseta	SMX
	Spionida	Spionidae	*Polydora socialis*	FDT
			Spio filicornis	SDT
			Prionospio steenstrupi	SDT
		Cirratulidae	*Chaetozone setosa*	SDT
		Apistobranchidae	*Apistobranchus typicus*	FDT
	Opheliida	Opheliidae	*Ophelina breviata*	BMX
	Orbiniida	Paraonidae	*Aricidea catherinae*	BMX
			Aricidea wassi	BMX
	Phyllodocida	Phyllodocidae	*Eteone longa*	CMX
		Polynoidae	Polynoidae juvenile	C(D/M)J

Note: Guild refers to feeding guilds of polychaetes defined by Fauchald and Jumars (1979). S, surface deposit feeder; F, filter feeder; B, burrower; C, carnivore; M, motile; D, discretely motile; X, nonjawed; T, tentaculate; J, jawed.

high critical probability. Yet the overall conclusion of the study is that there was a dearth of statistically significant effects of trawling. That outcome cannot be an artefact of either the inadequacies of the sampling design or the choice of α. It does of course remain true that the spatial variability of the benthos, coupled to the necessarily limited number of samples, resulted in many of the tests having low statistical power. The general lack of statistical significance may, therefore, have resulted from Type II errors. However, it is notable that the experiment was capable of detecting natural interannual variability, indicating that any real effect of the trawling on the macrofauna is, in comparison, minor.

The 12 passes of an otter trawl down the same track within 1.5 days, as in this experiment, represented a high intensity of fishing by typical commercial standards. However, that level of impact was not continued through a prolonged fishing season, nor were adjacent areas of seabed fished as they would be in commercial operations. Thus, even small benthic animals could readily recolonize the trawl track by transport over a few metres, a mechanism that might not so readily operate following widespread fishing (although commercial trawling is not uniformly distributed in space and will invariably leave some unfished patches as sources for recolonization). Perhaps more importantly, there is no evidence that the videograb ever sampled in the narrow track of an otter board (<1 m wide), but some probably did. Thus, the impacts observed are primarily those of the trawl's rockhopper footgear, sweeps, and belly meshes. Yet, previous studies suggest that the major seabed impacts of trawling result from the action of the boards (e.g., Rumohr and Krost 1991; Gilkinson et al. 1998). Finally, initial abundance and the timing and duration of impacts relative to the reproductive season of the organisms are likely to be important factors in determining the magnitude and direction of trawling impacts. Considering all of these issues, the results of the present experiment should not be uncritically extrapolated to the impacts of commercial trawling.

The presence of bioturbators in the study area, such as sand dollars and some polychaetes, opens the potential for substantial changes in community structure associated with trawling-induced changes in their abundance. The actions of these bioturbators provide a habitat complexity that can be critical to the maintenance of species diversity in unconsolidated sediments (Steimle 1989; Thistle and Eckman 1990). Sand dollars in particular are considered to be critical in structuring sandy-bottom benthic communities. Their movement and burrowing activity particularly affect tube-dwelling polychaetes and the meiofauna (Brenchley 1981). The resulting sediment disturbance is ranked second only to the effects of major storms in determining macrofaunal community structure on Sable Island Bank, off Nova Scotia, Canada (Stanley and James 1971). While many polychaetes have potential for rapid recovery, sand dollars have an average life span of 8 years and are unable to survive damage to their tests. The immediate loss of 37% of the sand dollar biomass after trawling in 1994 is therefore of considerable concern as an indicator of possible consequences of commercial-scale otter trawling. Future studies should record data on empty shells and dead tests in order to assess cumulative effects.

In this study, 12 taxa, representing eight families and five orders of the Polychaeta, appeared to have particularly dynamic populations in response to physical disturbance whether caused by trawling or by natural phenomena (Table 7). They included *Chaetozone setosa* and four other sedentary filter- or deposit-feeding spionids, which tend to be small and short-lived (<2 years), with possibly more than one recruitment period per year (Fauchald and Jumars 1979; Hily 1987). Four were errant burrowers of the families Opheliidae, Paraonidae, and Phyllodocidae, plus juveniles of the equally motile polynoids, and two capitellid deposit feeders, one of which is a tube-building species. Species such as these that are naturally variable from year to year may show the greatest responses to fishing-induced disturbance. However, they cannot be used as indicators of trawling impacts, except in carefully designed experiments, because their natural variations will always be confounded by the effects of trawling. Thus, the expensive and demanding experimental approach to studies of seabed impacts of fishing gear will continue to be needed.

The overall conclusion of this experiment, that the effects of otter trawling on the infauna were limited and short term,

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is in accord with some previous studies, at least when the gear was towed on sandy bottoms lacking prominent bedforms and when the effects of the otter boards were ignored (e.g., Gibbs et al. 1980; Hall et al. 1993; Simboura et al. 1998). This conclusion is, however, specific to the conditions of this study, including its emphasis on the infauna and macroepifauna. More extensive, commercial-scale trawling may yet prove to have substantial impacts even on the infauna, particularly if vulnerable species such as sand dollars have key roles in shaping community structure and function.

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Biotic influences on habitat selection by young-of-year walleye (*Stizostedion vitreum*) in the demersal stage

Thomas C. Pratt and Michael G. Fox

Abstract: The influence of prey availability and predation risk on the distribution of young-of-year (YOY) walleye (*Stizostedion vitreum*) was investigated by comparing species associations with the relative abundance of YOY walleye across nine habitat types using an underwater visual assessment technique. During the early demersal period (mid-June to mid-July), YOY walleye were found primarily in areas of high macrophyte cover at 2–5 m depth. YOY walleye abundance was positively correlated with the abundance of prey fishes at this time. YOY walleye shifted to low-cover, shallow areas during the late demersal period (mid-July to late August), and the significant prey associations disappeared. Although the selected habitats are considered to have low predation risk, the distribution of YOY walleye was not related to our index of predator abundance in either time period. YOY walleye were not observed in three of the nine habitat types, suggesting that active habitat selection was occurring. High macrophyte cover and prey availability appear to be the major factors influencing habitat selection during the early demersal period. Although our results do not demonstrate the functional significance of the shift of YOY walleye into shallow water, we hypothesize that these habitats are selected as refugia from particular predators such as adult walleye.

Résumé : Une comparaison des associations d'espèces et de l'abondance des jeunes Dorés (*Stizostedion vitreum*) de l'année (YOY) dans neuf types d'habitat à l'aide d'une technique d'inventaire visuel sous-marin a permis d'évaluer l'influence de la disponibilité des proies et du risque de prédation sur la répartition des dorés YOY. Au début de la phase démersale (mi-juin à mi-juillet), les dorés YOY se retrouvent principalement dans des zones à forte couverture de macrophytes à une profondeur de 2–5 m; à cette période, leur abondance est en forte corrélation avec la densité des poissons qui leur servent de proies. Plus tard dans la phase démersale (mi-juillet à la fin d'août), les dorés YOY se déplacent vers des zones peu profondes à faible couverture végétale et l'association significative avec les proies disparaît. Bien que les habitats étudiés semblent présenter un faible risque de prédation, la répartition des dorés YOY n'est pas reliée à notre indice d'abondance des prédateurs ni à l'une ni à l'autre des périodes. Les dorés YOY n'ont pas été observés dans trois des neuf types d'habitat, ce qui laisse croire qu'il existe une sélection active des habitats. Une forte couverture de macrophytes et la disponibilité des proies semblent être les facteurs déterminants du choix de l'habitat durant la première période démersale. Bien que nos résultats n'expliquent pas la signification fonctionnelle du déplacement des dorés YOY vers les eaux moins profondes, nous posons l'hypothèse que ces habitats sont choisis comme refuges contre certains prédateurs, comme les dorés adultes.

[Traduit par la Rédaction]

Introduction

Early life history paradigms suggest that juvenile fishes should attempt to maximize growth rates, as mortality decreases with increasing body size (Houde 1987). Thus, one might expect juvenile fishes to reside in habitats that provide the greatest potential for growth. However, size-selective predation has been identified as one of the primary mechanisms behind size-selective mortality in juvenile fishes (review by Sogard 1997), and habitats with the highest potential for growth often have the highest predation risk (Werner et al. 1983). Typically, habitats with high structural complexity tend to have lower predation rates, as they provide more refuge opportunity for prey fishes (Savino and Stein 1982; Werner et al. 1983). Therefore, juvenile fishes have been predicted to select habitats that minimize mortality in relation to foraging return (Gilliam and Fraser 1987). For many juvenile freshwater fishes, this means utilizing suboptimal foraging habitats or altering foraging behaviour in order to reduce predation risk to an acceptable level (Werner et al. 1983; Abrahams and Dill 1989).

A general pattern of risky life stages residing in structurally complex habitats is apparent in a number of fish species (Werner et al. 1983; Rozas and Odum 1988; Eklöv 1997). However, there are some species that appear to favour areas

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of high prey availability over refuge (Leis and Fox 1996) and others that have both high prey availability and low predation risk in the same habitat (Rozas and Odum 1988).

Species that grow quickly face a series of transitional ecological requirements early in their development. This is undoubtedly true for young-of-year (YOY) walleye (*Stizostedion vitreum*), which undergo a series of ontogenetic shifts during their first year of life. Although the exact timing of the ontogenetic shift varies by waterbody, YOY walleye ultimately become demersal and piscivorous. In one particularly well-studied population, this transformation usually occurs in late June (Houde and Forney 1970). YOY walleye then grow rapidly, often achieving lengths in excess of 200 mm in their first year of life (Scott and Crossman 1973). Piscivores such as walleye, that face a number of shifts in ontogeny, compete with species early in their life history that they will eventually prey upon (Werner and Gilliam 1984). The requirements of such a relatively complex life history are assumed to constrain piscivores into being poor competitors during their early life stages (Werner and Gilliam 1984); therefore, piscivores may be more likely to favour risky habitats because they are particularly sensitive to the benefits of rapid growth.

To date, the lack of habitat preferences identified for YOY walleye has led researchers to conclude that young walleye are habitat generalists, with no specific preferences after the commencement of the demersal stage. For example, studies on riverine populations have shown that YOY walleye are very flexible in their early habitat choice (Leis and Fox 1996) and that these generalist tendencies continue until late summer. YOY walleye were almost ubiquitous in a Bay of Quinte survey by Savoie (1983), although sandy sites produced the greatest numbers in other Ontario lakes (Ritchie and Colby 1988). The only trend that is apparent in these studies is that YOY walleye are not typically found in heavily vegetated sites, probably because such sites are used by ambush predators such as largemouth bass (Santucci and Wahl 1993). This notion is supported by a negative correlation between the survival of stocked walleye fingerlings and the extent of broad, leafy macrophyte cover within a waterbody (Seip 1995).

There are several possible explanations for the apparent habitat generalist tendencies of young walleye. One possibility is that the distribution of YOY walleye may be influenced by predator–prey interactions. Leis and Fox (1996) found that YOY walleye in a northern Ontario river were more closely associated with their prey than with any particular habitat type. This observation would support the suggestion that walleye favour areas of high prey density over refuge habitats in order to facilitate rapid early growth and would also explain the habitat generalist tendencies of young walleye. As walleye recruitment and growth were found to be regulated by the availability of suitable prey items, and the switch to piscivory is critical for YOY walleye survival (Forney 1976), it seems possible that food requirements could force young walleye to act as habitat generalists, especially if the preferred prey species are located in many different habitats.

A second possibility may involve the sampling techniques traditionally used for YOY walleye collection. These include seines, trawls, and electrofishers (e.g., Savoie 1983; Leis and Fox 1996), which generally sample at a spatial scale that is too broad to allow the determination of microhabitat preference. Species microhabitat preferences are most effectively sampled with visual techniques (Sale 1980), although relatively few researchers have used underwater methods to determine fish habitat preferences in freshwater systems. Direct underwater observations are not without fault, as they typically underrepresent cryptic and pelagic species (Brock 1982). However, all sampling techniques possess unique biases, and few techniques are capable of quantifiably assessing both potential predator and prey species across a variety of habitat types (T.C. Pratt and M.G. Fox, unpublished data).

The goals of this study were to assess habitat selection of YOY walleye in a lacustrine environment and to determine whether habitats used by young walleye are related to the distribution of their predators or prey. The primary objective of this study was to test hypotheses about walleye distribution during the demersal phase of their early life history, a critical period that can strongly influence recruitment variability in this species (Forney 1976). We hypothesized that (*i*) YOY walleye would be habitat generalists, (*ii*) their distribution would be positively related to that of their prey, and (*iii*) their distribution would be negatively related to that of their predators. Our secondary objective was to examine the shoaling patterns of young walleye in these habitats, in particular to determine the species composition of shoals that included young walleye and whether there were changes in shoaling patterns that accompanied habitat shifts.

Materials and methods

Study site

This study was performed on Big Clear Lake (44°43′W, 76°55′N), a 337-ha waterbody located near the town of Arden, Ontario, Canada. The surrounding basin is typical of the Precambrian Shield formation, with rock outcroppings and thin pockets of sandy soil. Big Clear Lake is a headwater lake fed by two major inflow streams, with one drainage outlet. The lake itself consists of a number of large bays, roughly divided into four interconnected basins. Irregular glacial scouring has led to the formation of numerous small islands and shoals located throughout the lake, resulting in a large and diverse littoral zone. The combination of high habitat diversity and a strong, naturally reproducing walleye population makes Big Clear Lake an excellent lake for this study.

Big Clear Lake is relatively shallow (mean depth = 6.6 m) and thermally stratified from May to November. Water quality parameters for Big Clear Lake are typical of a mesotrophic waterbody in Canada (means of point samples taken at 30 sites during the study: pH = 8.1, conductivity = 248 $\mu S \cdot s^{-1}$, total phosphorus = 68 $\mu g \cdot L^{-1}$). Surface water temperature ranged from 18 to 26°C during the study, while Secchi depths ranged from 3.5 to 4.1 m.

Habitat assessment and classification procedure

YOY walleye habitat preference and predator and prey associations were assessed using a modification of the rapid visual technique (RVT) introduced by Jones and Thompson (1978). The RVT was developed as an alternative to the straight-line underwater transect, with divers searching a predetermined area for a specific length of time (Jones and Thompson 1978). Any species observed during an RVT are assigned a score based on first observation time, thus allowing relative abundance to be estimated. In effect, the RVT substitutes time for area during a search while assuming that the most abundant species will be observed early in a trial.

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Table 1. Body length criteria used for classifying species life stages observed by the RVT (based on Scott and Crossman 1973).

	Length (mm)		
Species	YOY	Juvenile	Adult
Northern pike (*Esox lucius*)	<150	151–299	>300
Blackchin shiner (*Notropis heterodon*)	—	<30[a]	>30
Mimic shiner (*Notropis volucellus*)	—	<30[a]	>30
Bluntnose minnow (*Pimephales notatus*)	—	<35[a]	>35
Golden shiner (*Notemigonus crysoleucas*)	—	<50[a]	>50
Brown bullhead (*Ameiurus nebulosus*)	—	<50[a]	>200
Banded killifish (*Fundulus diaphanus*)	—	<40[a]	>40
Pumpkinseed (*Lepomis gibbosus*)	<30	31–109	>110
Bluegill (*Lepomis macrochirus*)	<30	31–119	>120
Smallmouth bass (*Micropterus dolomieu*)	<45	46–249	>250
Largemouth bass (*Micropterus salmoides*)	<50	49–249	>250
Rock bass (*Ambloplites rupestris*)	<40	41–199	>200
Yellow perch (*Perca flavescens*)	<40	41–139	>140
Walleye (*Stizostedion vitreum*)	<180	181–299	>300
Logperch (*Percina caprodes*)	—	<35[a]	>35

[a]YOY not separated from older juveniles, either because this could not be done easily by underwater observation or because no YOY were observed.

Table 2. Habitat classification scheme developed to separate habitats into discrete entities.

	Substrate/cover				
Depth (m)	Rock	Bare	*Chara*/*Najas*	15% cover	>30% cover
0–2	SR (shallow, rock)	SMu (shallow, mud)	SC (shallow, *Chara*)	SMi (shallow, medium cover)	SV (shallow, vegetated)
2–5	—	—	MC (medium depth, *Chara*)	MMi (medium depth, medium cover)	MV (medium depth, vegetated)
5–7	—	—	DC (deep, *Chara*)	—	—

Note: Habitat categories are based on depth, substrate, and percent macrophyte cover.

The original the RVT of Jones and Thompson (1978) was modified as follows. Nine predefined habitats were treated as distinct areas, like the coral reefs of the Jones and Thompson (1978) study. Since Big Clear Lake, like most temperate waterbodies, is species depauperate relative to tropical coral reefs, the largest change required to adapt the RVT to our study was to shorten the length of a trial. As Big Clear Lake was thought to contain approximately one tenth the number of species found by Jones and Thompson (1978), we used a 5-min period for our trials, as opposed to their 50-min trials. Species were then assigned scores based on what minute they were first observed in. For example, a species seen in the first minute would be assessed five points, and a species first observed in the fifth minute would be assigned one point. This scoring system is analogous to the original RVT method, where a species observed in the first 10 min of the 50-min trial received five points, and a species first observed in the last 10-min period would receive one point (Jones and Thompson 1978). Where possible, fish were identified to life stage as well as to species, based on the classification displayed in Table 1.

For this study, a habitat classification scheme was developed based on depth, substrate, and percent macrophyte cover (Table 2). The result was five habitat types located in shallow water (0–2 m), three at middepths (2–5 m), and one in deeper water (5–7 m). At middepths, the muddy and rocky sites found in shallower water disappeared, whereas all vegetation except the colonial algae *Chara* spp. stopped growing at a depth of 5 m. Preliminary trials were attempted at depths below 7 m, but no fish were ever observed. Percent cover estimates were based on the amount of submerged macrophyte cover calculated from 1-m^2 quadrats. In Big Clear Lake, the dominant aquatic macrophytes are *Potamogetan* spp. and Eurasian watermilfoil (*Myriophyllum spicatum*). *Chara* spp. provided some level of cover, and because it grows in extensive monotypic mats in Big Clear Lake, it was considered a separate habitat type.

RVT trials were conducted during daylight hours from June 15 to August 21, 1999, at 401 sites distributed throughout the lake. Sites are defined here as continuous areas of relatively uniform habitat, ranging in area from 350 m^2 to approximately 1000 m^2. Shallow habitats were assessed with snorkelling, whereas SCUBA was used for all middepth and deep trials. Observers swam slowly throughout the 5-min trial, typically covering areas ranging from 80 to 120 m^2. Observers were equipped with a water-resistant watch and a white PVC wrist slate to record the time when species and life stages were first seen in each trial.

Four sequential replicate RVTs were performed at each site on the day that site was assessed. This number was based on a preliminary assessment of 33 sites representing all nine habitat types, which showed that on average, 95% of the life stages recorded in six to eight within-site trials were observed by the fourth trial (range by habitat type = 86–100%.) Scores from replicate counts were averaged, giving a single RVT score for each site. Habitat types were sampled in approximate proportion to their availability, and the order of sampling was randomized over the period of the study. The total number of sites assessed in a given habitat type ranged from 41 to 50.

In order to assess YOY walleye prey composition, 10 individu-

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als were captured with seines every 2 weeks, starting July 15, for stomach content analysis. Prior to this date, we were unable to capture YOY walleye due to their physical location. The first YOY walleye observation was on June 15, and as YOY walleye become piscivorous shortly after becoming demersal (Raney and Lachner 1942; Dobie 1966), it was assumed that walleye were eating piscine prey in this 1-month period when they could be observed but not captured. Collected walleye were taken back to the laboratory where prey were removed from the stomachs. Identifiable prey were classified to species and life stage and their lengths were measured. The lengths of partially digested prey that could not be identified were determined by comparing the remaining body parts with those of a prey item of known length.

Assessment of YOY walleye habitat preference and species associations

Walleye abundance scores obtained from RVT trials were used to assess YOY walleye habitat preferences and predator and prey associations. As most fishes undergo a series of ontogenetic shifts (Werner and Gilliam 1984), we first examined the data for evidence of temporal habitat shifts by performing a two-way analysis of variance (ANOVA) on ranked walleye abundance data (Zar 1999). Nonparametric tests were used in all analyses involving walleye abundance scores due to the large number of trials where YOY walleye were not observed. As a result, these data could not be transformed to meet the assumptions for parametric tests. A significant shift in habitat use was apparent after the first 4 weeks of the study (Table 3). As a result, the study period was divided into early (June 15 – July 11) and late (July 15 – August 21) demersal phases, and all hypotheses were tested in each phase.

To assess whether walleye exhibited any habitat preferences, walleye abundance scores in the nine habitat types were compared with the Kruskal–Wallis one-way ANOVA (Zar 1999). When significant differences in habitat use were found, Dunn's post hoc test was performed to determine which habitats differed.

Shifts in YOY walleye distribution were also examined by comparing their spatial distribution at two levels of vegetation and depth in the early and late demersal periods. For this analysis, habitats with little or no YOY walleye utilization were excluded, and changes in depth and vegetation usage by YOY walleye over the two time periods were examined by performing a two-way ANOVA on ranked walleye abundance data.

RVT scores for all observed species and life stages were compared with YOY walleye RVT scores from the same sites to determine whether YOY walleye were positively or negatively associated with any particular species or life stage. RVT scores were divided into the early and late demersal period, and Spearman rank correlations were used to test for species associations. Correlations were Bonferroni adjusted by dividing the desired probability level by the number of pairwise comparisons (Zar 1999).

Assessment of prey availability and predation risk on YOY walleye habitat selection

Associations between sites and habitats selected by YOY walleye and the abundance of their prey and predators were examined by first developing indices of potential prey and predator density and then using these indices to assess the associations. The prey abundance index (PREYIND) was based on YOY walleye stomach content data obtained from this study and supplemented by YOY walleye prey data from lakes with similar prey communities (Raney and Lachner 1942; Maloney and Johnson 1957; Dobie 1966). In natural systems, YOY walleye have been found to forage primarily on other percids (Raney and Lachner 1942; Dobie 1966; Lyons 1987), while laboratory studies have found that young walleye are more likely to select soft-bodied (cyprinid) prey (Campbell 1998). Thus, for the early demersal period, PREYIND was defined as the sum of the RVT scores for the following species life stages: YOY blackchin shiner, YOY banded killifish, YOY bluntnose minnow, YOY golden shiner, YOY *Lepomis* spp., YOY largemouth bass, YOY and adult mimic shiner, YOY yellow perch, and unidentifiable fry. For the late demersal period, PREYIND was identical to that of the early period except that blackchin shiner adults were added to the prey list. Blackchin shiner adults were too large to be eaten by YOY walleye in the early demersal period.

Table 3. Results of a two-way ANOVA on the ranked RVT scores investigating potential shifts in YOY walleye habitat use between the early and late demersal periods.

Source of variation	df	F	P
Period	1, 383	1.1	0.29
Habitat	8, 383	3.2	0.002
Period × habitat	8, 383	4.6	<0.001

The predator abundance index (PREDIND) was developed similarly using potential YOY walleye predators for both the early and late demersal periods in separate indices. Only species defined as active piscivores by Scott and Crossman (1973) and large enough to consume YOY walleye during the early demersal period, based on a prey to predator length ratio of 0.4 (Juanes 1994), were included in the analysis. For the early demersal period, PREDIND was defined as the sum of the RVT scores for all yearling and older northern pike, largemouth bass, smallmouth bass, yellow perch, and walleye. Due to rapid YOY walleye growth, a number of piscivore life stages were no longer capable of consuming young walleye in the late demersal period based on the prey to predator length ratio of 0.4. The remaining life stages used to compose PREDIND in the late demersal period were yearling and older northern pike, adult largemouth and smallmouth bass, and adult walleye.

Spearman rank correlations between the prey or predator indices and the YOY walleye RVT scores were used to test the strength of predator and prey associations across habitats. YOY walleye scores were correlated with PREYIND and PREDIND, with the early (127 sites) and late (274 sites) demersal periods examined separately. In order to determine whether the distribution of YOY walleye could potentially be explained by differences in prey availability or predation risk among habitat types, Kruskal–Wallis ANOVAs were performed to compare PREYIND and PREDIND among habitat types, followed by Dunn's post hoc test to identify which habitats differed.

Finally, PREYIND and PREDIND were used to compare the role of relative prey availability and predation risk on microhabitat selection within the most frequented habitats. To accomplish this, Student's t tests were used to compare PREYIND and PREDIND from sites where YOY walleye were observed with those from sites in the same habitat type where YOY walleye were not observed.

Assessment of YOY walleye shoaling behaviour

Whenever a YOY walleye was observed during an RVT trial, the species and number of individuals shoaling with YOY walleye were recorded. Differences in shoaling behaviour between the early and late demersal periods were examined by comparing the average shoal size and the number of YOY walleye in each shoal. Both tests were performed on $\log_e$-transformed data with Student's t tests. Species associations were also compared across time periods by determining the number of times that YOY walleye were observed schooling with a particular species during each time period and using Fisher's exact test to determine differences in species shoaling with YOY walleye across time periods.

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Fig 1. YOY walleye habitat use in the (*a*) early and (*b*) late demersal periods, as indicated by RVT scores in nine habitat types. Error bars represent standard error. Means with the same letter within each period are not significantly different. SR, shallow, rock; SMu, shallow, mud; SC, shallow, *Chara*; SMi, shallow, medium cover; SV, shallow, vegetated; MC, medium depth, *Chara*; MMi, medium depth, medium cover; MV, medium depth, vegetated; DC, deep, *Chara*.

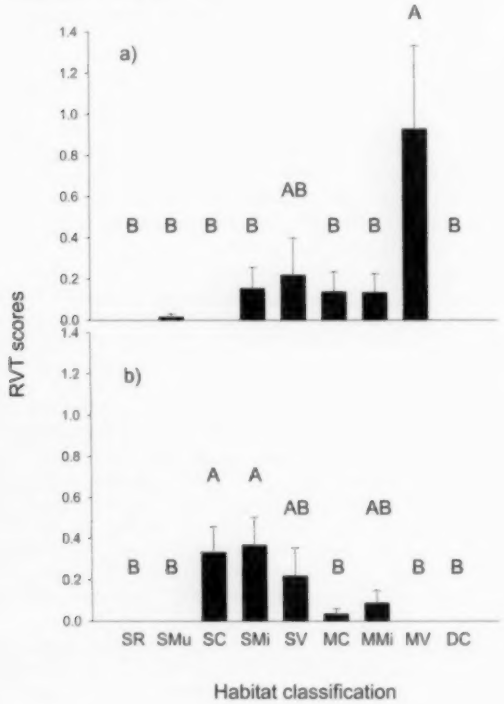
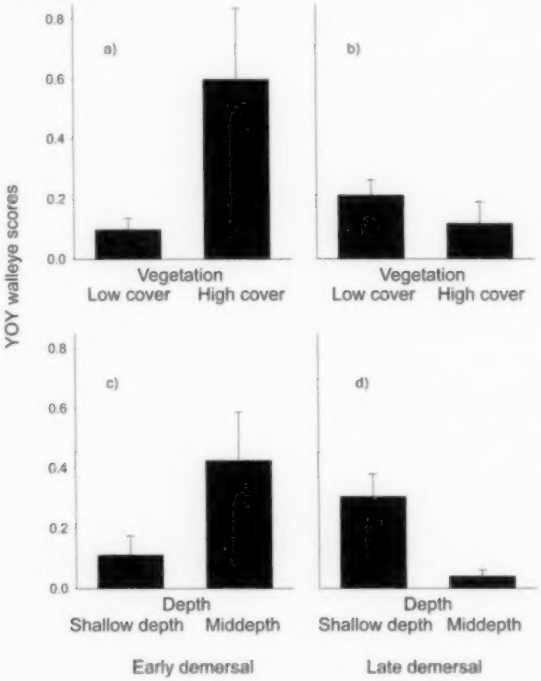


Fig. 2. Comparison of YOY walleye RVT scores by (*a* and *b*) vegetation cover and (*c* and *d*) depth during the early and late demersal periods. Error bars represent standard error.



Results

Habitat use

Both the early and late demersal periods showed significant differences among habitats in their use by YOY walleye (early demersal: $H_8 = 20.2$, $P = 0.01$; late demersal: $H_8 = 41.2$, $P < 0.001$). During the early demersal period, YOY walleye were found at significantly higher abundances in heavily vegetated medium-depth habitats (Fig. 1*a*). Four other habitats were used at intermediate levels during the early demersal period, while the remaining four habitats were rarely or never utilized. During the late demersal period, shallow *Chara* and shallow habitats with moderate cover showed significantly higher levels of use than five of the other seven habitat types, including four in which YOY walleye were not observed (Fig. 1*b*).

The shift in habitat use across the demersal periods, previously described as the basis for the temporal separation of the data, was evident from the change in relative usage of the various habitats in the two time periods (Fig. 1; Table 3). In particular, heavily vegetated medium-depth habitats had high usage during the early demersal phase but no usage during the late demersal phase, while shallow *Chara* habitats displayed the opposite pattern. Three habitat types, shallow rock, shallow mud, and deep *Chara*, were rarely or never utilized in either phase.

A similar pattern is observed upon reducing YOY walleye habitat types into two depth and cover regimes. Significant time by cover and time by depth interactions ($P < 0.001$ in both cases) indicated that there was a shift in cover and depth utilization between the two periods. YOY walleye appeared to move away from middepth, high-cover habitats towards shallow, low-cover habitats as they grew older and larger (Fig. 2).

Prey and predator associations

Stomach content analysis indicated that YOY walleye were almost entirely piscivorous by the end of the early demersal period (Table 4). Unfortunately, only a few of the walleye collected during this period contained identifiable prey items in their stomachs. Most of these were YOY fishes, although an adult cyprinid was also observed. The unidentifiable fishes were also mostly YOY.

Twenty-seven YOY walleye were collected in the late demersal period, and the stomach contents of the 21 that contained prey items consisted entirely of fish. The most common prey types were YOY sunfish, but five other species were identified, including an adult mimic shiner.

YOY walleye RVT scores showed a significant, positive correlation with the RVT scores of three life stages during the early demersal period and six life stages during the late demersal period (Table 5). Based on stomach content data, the three species associated with YOY walleye during the early demersal period (YOY bluntnose minnow, mimic shiner

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Table 4. Stomach contents of YOY walleye captured in Big Clear Lake during the summer of 1999.

Prey type	Total number of prey type found	Percentage of walleye with prey type	Mean percent volume
Early demersal period (n = 10 walleye; mean length = 77 mm ± 2.4 SE)[a]			
Mimic shiner adults	1	10	11
YOY bluntnose minnow	1	10	3
YOY *Lepomis* spp.	2	20	13
Unidentified fish remains	8	60	71
Chironomid larvae	1	10	2
Late demersal period (n = 27 walleye; mean length = 106 mm ± 2.7 SE)[b]			
Mimic shiner adults	1	4	2
YOY mimic shiner	3	4	1
YOY bluntnose minnow	4	7	4
YOY banded killifish	1	4	2
YOY *Lepomis* spp.	13	22	20
Logperch	1	4	1
YOY yellow perch	5	15	9
Unidentified fish remains	32	53	61

[a]Total number of walleye examined in early demersal period includes two with empty stomachs. Mean (±SE) length of fish prey in the early demersal period was 11.9 ± 0.8 mm.
[b]Total number of walleye examined in late demersal period includes six with empty stomachs. Mean (±SE) length of fish prey in the late demersal period was 19.3 ± 1.2 mm.

adults, and YOY yellow perch) were all potential prey, despite the fact that YOY yellow perch and mimic shiner adults were often seen in loose shoals with YOY walleye at this time. In the late demersal period, none of the life stages significantly associated with YOY walleye (bluntnose minnow adults, golden shiner adults, largemouth bass juveniles, juvenile pumpkinseed older than age 1, pumpkinseed adults, and walleye yearlings) were considered potential predators or prey. YOY walleye were observed shoaling with most of these species (particularly golden shiner adults). A significant correlation was found between the YOY walleye RVT score at a site and its PREYIND score during the early demersal period (r_s = 0.36, n = 127, P < 0.001) but not during the late demersal period (r_s = –0.057, n = 274, P = 0.35).

Significant differences in prey availability were detected among habitats (early demersal: H_8 = 25.6, P < 0.001; late demersal: H_8 = 37.5, P < 0.001). Results from a Dunn post hoc test indicated that medium and deep habitats had significantly greater prey abundance than four of the five shallow habitats in both the early and late demersal periods. Most of the YOY walleye were found in four of the habitats with the highest prey abundance during the early demersal period (Fig. 3*a*). Both the PREYIND and the YOY walleye RVT score were highest in middepth vegetated habitats and second highest in shallow vegetated habitats. This association disappeared during the late demersal period (Fig. 3*b*), as the habitats used most frequently had among the lowest prey availability.

When the habitat types most frequented by YOY walleye were examined individually, it was found that prey abundance was consistently higher at sites where walleye were observed than at sites where they were not observed (Table 6). In particular, vegetated sites at medium depths (preferred during the early demersal period) and shallow sites with moderate cover (preferred during the late demersal period) with YOY walleye had significantly higher PREYIND scores than sites of the same habitat type where YOY walleye were not found. This trend was also apparent in shallow *Chara* habitats, although the difference between sites with and without YOY walleye was not significant.

YOY walleye RVT scores were negatively associated with those of a number of potential predators, but no significant relationships were observed (Table 5). Contrary to our prediction, the walleye RVT score at a site was not significantly correlated with its PREDIND during the early (r_s = 0.02, n = 127, P = 0.78) or late (r_s = 0.02, n = 274, P = 0.73) demersal periods.

During the early demersal period, the PREDIND differed significantly among habitats (H_8 = 42.4, P < 0.001), and a Dunn post hoc test indicates that predators were less numerous in shallow, muddy habitats than in the other eight habitat types. However, no evidence for avoidance behaviour in YOY walleye was detected, as they too were rarely found at these muddy sites (Fig. 4*a*). In the late demersal period, the PREDIND scores also differed significantly among habitat types (H_8 = 43.1, P < 0.001). A Dunn post hoc test indicated that vegetated habitats at medium depth contained higher numbers of predators than all of the shallow habitat types (Fig. 4*b*). When the three habitats most frequented by YOY walleye were examined individually, it was found that sites where YOY walleye were found did not differ significantly in predator abundance from sites where they were not found (Table 6).

Shoaling behaviour

There were significant differences between the early and late demersal periods in the size of shoals containing YOY walleye, the number of YOY walleye shoaling together, and the species composition of the shoals (Table 7). YOY walleye were associated with larger, mixed-species shoals in the early demersal period, but later, they tended to shoal in smaller, more homogeneous groups. Species associations within the shoals also shifted between periods, as YOY yellow perch were commonly found shoaling with walleye

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Table 5. Spearman rank correlations between YOY walleye RVT scores and those of other species by life stage.

		Early demersal (n = 127)		Late demersal (n = 274)	
Species	Life stage	r_s	P	r_s	P
Northern pike	Adult	–0.07	0.46	–0.06	0.37
	Juvenile	—	—	–0.04	0.53
Blackchin shiner	Adult	—	—	–0.08	0.17
	YOY	—	—	–0.02	0.75
Mimic shiner	Adult	0.30	<0.001*	0.12	0.04
	YOY	—	—	–0.09	0.12
Bluntnose minnow	Adult	0.25	0.005	0.24	<0.001*
	YOY	0.28	<0.001*	–0.03	0.58
Golden shiner	Adult	–0.04	0.66	0.37	<0.001*
	YOY	—	—	–0.001	0.98
Brown bullhead	Adult	0.10	0.26	–0.07	0.29
	YOY	–0.03	0.72	—	—
Banded killifish	Adult	–0.09	0.32	0.04	0.51
	YOY	—	—	–0.04	0.48
Pumpkinseed	Adult	0.09	0.31	0.20	0.001*
	Juvenile	0.07	0.42	0.24	<0.001*
Bluegill	Adult	0.07	0.45	0.03	0.64
	Juvenile	0.14	0.11	0.16	0.006
Lepomis spp.	YOY	0.16	0.08	–0.11	0.08
Smallmouth bass	Adult	–0.16	0.08	–0.11	0.07
	Juvenile	–0.15	0.10	–0.09	0.14
	YOY	–0.12	0.17	–0.11	0.06
Largemouth bass	Adult	0.05	0.58	0.04	0.52
	Juvenile	0.11	0.20	0.23	<0.001*
	YOY	0.10	0.27	0.08	0.17
Rock bass	Adult	0.02	0.82	0.06	0.36
	Juvenile	0.02	0.80	–0.06	0.36
Yellow perch	Adult	–0.04	0.70	0.19	0.002
	Juvenile	0.08	0.37	0.18	0.003
	YOY	0.27	0.002*	–0.05	0.38
Walleye	Adult	0.09	0.31	–0.05	0.44
	Yearling	0.01	0.94	0.27	<0.001*
Logperch	Adult	–0.03	0.74	–0.12	0.06
	YOY	–0.08	0.37	–0.15	0.01

Note: Individual probabilities are reported; asterisks indicate statistical significance ($P < 0.05$) after applying Bonferroni corrections.

during the early demersal period but not during the late demersal period. Adult golden shiner exhibited the reverse trend, appearing in shoals with YOY walleye primarily in the late demersal period. Only adult mimic shiner were consistently shoaling with young walleye in both time periods.

Discussion

Although biotic and abiotic factors appeared to play a role in determining the distribution of YOY walleye in Big Clear Lake, the relative importance of physical habitat features and biotic interactions differed temporally. For the first few weeks of the demersal stage, the distribution of YOY walleye was positively related to prey availability, and walleye were found most frequently at sites of moderate depth and moderate to dense macrophyte cover. A positive relationship between the abundance of YOY walleye and their prey was predicted, as a similar relationship was noted in an Ontario river system (Leis and Fox 1996). The loss of a significant association with prey in the late demersal period was unexpected, as high prey levels were found to be associated with YOY walleye at least through the end of July by Leis and Fox (1996). YOY walleye distribution was not correlated with our PREDIND in either demersal period. Several possible explanations exist for the shifting balance between prey associations and habitat preference in YOY walleye habitat selection and the apparent lack of influence of predation risk.

Habitat use

The early demersal period extended from mid-June to mid-July, and at this time, YOY walleye were located primarily at heavily vegetated sites 2–5 m in depth. These sites consisted mostly of thick stands of Eurasian watermilfoil, with one or two individual walleye mixed in shoals along with hundreds of adult mimic shiner and YOY yellow perch. During this period, YOY walleye were rarely found in habi-

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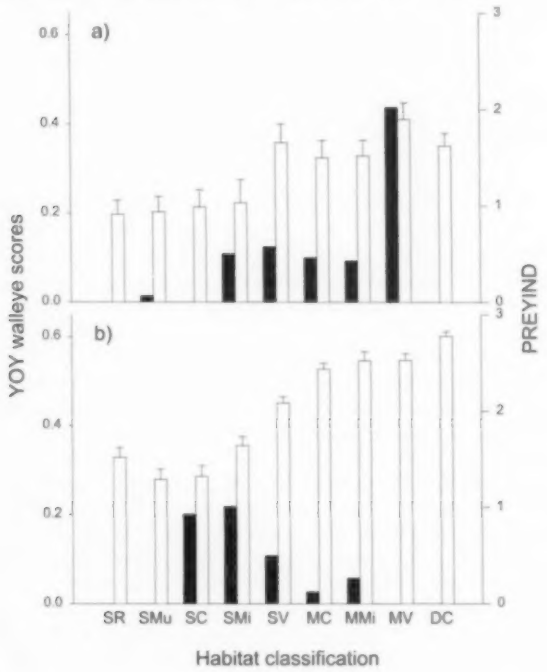
Fig. 3. Comparison of YOY walleye habitat use patterns (solid bars) and prey abundance (open bars), as indicated by the PREYIND, in the nine habitat types defined in this study in the (*a*) early and (*b*) late demersal periods. Prey abundance error bars indicate standard error. SR, shallow, rock; SMu, shallow, mud; SC, shallow, *Chara*; SMi, shallow, medium cover; SV, shallow, vegetated; MC, medium depth, *Chara*; MMi, medium depth, medium cover; MV, medium depth, vegetated; DC, deep, *Chara*.



tats that provided little or no cover. The utilization of heavily vegetated habitats was opposite to our predictions, as previous research had suggested that young walleye prefer more open habitats (Savoie 1983; Ritchie and Colby 1988; Lane et al. 1996) and should avoid vegetation to reduce the threat of largemouth bass predation (Santucci and Wahl 1993). It is possible that previous researchers who classified YOY walleye as habitat generalists may have missed the short time period where young walleye utilized high cover areas, as it occurred immediately after the pelagic phase and the fish were residing in areas difficult to sample using most traditional sampling gear.

Other studies have found YOY walleye to utilize vegetated habitats. Wahl (1995) found hatchery walleye more likely to reside in safer, high-cover areas than muskellunge (*Esox masquinongy*). Raney and Lachner (1942) reported difficulty in sampling YOY walleye in Oneida Lake, which were found almost exclusively in shallow macrophyte beds in the first week of August. Although the timing of this observation falls outside of our definition of the early demersal period, the growth rates of YOY walleye in Oneida Lake were slower than those observed in the present study, and the fish were similar in size to those found in vegetated habitats in Big Clear Lake. This suggests that the timing of the YOY walleye habitat shifts may be size rather than time dependent.

Considerable changes were observed in YOY walleye habitat preferences and prey associations after July 15. YOY walleye completely abandoned the middepth vegetated habitat and moved to shallower habitats with less available cover. The move to areas with reduced cover fits the traditional view of YOY walleye habitat selection (Savoie 1983; Ritchie and Colby 1988; Lane et al. 1996), but the selection of primarily shallow water (<2 m depth) suggests that YOY walleye may not be as affected by high light levels as older individuals (Ryder 1977). Other studies have found YOY walleye at depths of up to 10 m by the fall (Raney and Lachner 1942), and while the intensive component of this study ended in August, periodic SCUBA observations that extended into October indicated that most YOY walleye were still in shallow, low-cover habitats at that time.

Prey and predator associations

The apparent YOY walleye habitat preferences may be masking a greater dependence on prey availability during the demersal period. As predicted, a significant correlation was found between prey availability and YOY walleye abundance. In addition, three prey species were significantly correlated with YOY walleye abundance across all habitats during the early demersal period. These results, in combination with the occurrence of higher prey levels in vegetated habitats, suggest that the habitat preferences detected here may be prey related. Few studies have actually investigated the relative importance of prey and habitat associations in fishes, and the results have not been consistent. Some researchers have found strong habitat and weak prey associations (Perrow et al. 1996; Eklöv 1997), while others have found the opposite (Leis and Fox 1996; Muotka et al. 1998). The presence of both habitat and prey associations has been found in at least one other study (Rozas and Odum 1988). Both Rozas and Odum (1988) and Eklöv (1997) focussed on species targeted by piscivores, and both studies found the strongest habitat preference and highest prey availability in vegetated areas. Their results parallel those of our study and suggest that high prey availability is a factor in the selection of high-cover habitats by YOY walleye in the early demersal period.

The prey associations evident in the early demersal period ended by the start of the late demersal period. The significant relationship between YOY walleye and prey availability disappeared, and the species that were earlier found to be significantly correlated with YOY walleye abundance changed from potential prey to nonprey species of similar size that shoaled with the walleye. The loss of a significant association of YOY walleye with its prey was unexpected because this association extended through the month of July in the Montreal River (Leis and Fox 1996). One possible explanation is the difference between the two systems in productivity and prey availability, with Big Clear Lake being the more productive of the two. The combination of higher latitude and lower productivity in the Montreal River may have kept YOY walleye tied to their prey for a longer period in that system, and the shorter duration of the Leis and Fox (1996) study meant that YOY walleye may not have been sampled during the period when prey become less important.

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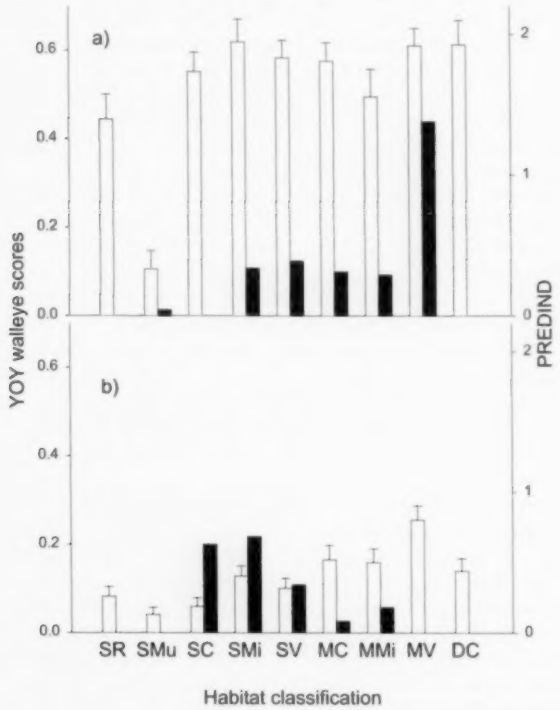
Table 6. Comparison of prey abandance (PREYIND) and predator abundance (PREDIND) between sites where YOY walleye were found and those where they were not found.

Period	Habitat	Walleye present	Walleye absent	*t*	df	*P*
PREYIND						
Early demersal	MV	8.5 (2.5)	4.4 (3.7)	2.7	13	0.01
Late demersal	SMi	6.1 (2.8)	3.6 (1.5)	2.4	28	0.01
Late demersal	SC	4.0 (1.8)	2.8 (1.4)	1.4	31	0.09
PREDIND						
Early demersal	MV	6.6 (2.8)	6.5 (7.2)	0.03	13	0.48
Late demersal	SMi	0.5 (0.06)	0.8 (0.17)	1.4	28	0.09
Late demersal	SC	0.3 (0.07)	0.3 (0.12)	0.3	31	0.41

Note: Comparisons were made in habitats most frequented by YOY walleye. Values in parentheses are the standard error of index scores. MV, medium depth, vegetated; SMi, shallow, medium cover; SC, shallow, *Chara*.

Fig. 4. Comparison of YOY walleye habitat use patterns (solid bars) and predator abundance (open bars), as indicated by the PREDIND,. in the nine habitat types defined in this study in the (*a*) early and (*b*) late demersal periods. Predator abundance error bars indicate standard error. SR, shallow, rock; SMu, shallow, mud; SC, shallow, *Chara*; SMi, shallow, medium cover; SV, shallow, vegetated; MC, medium depth, *Chara*; MMi, medium depth, medium cover; MV, medium depth, vegetated; DC, deep, *Chara*.



It is interesting to note that, despite the lack of a significant association between the abundance of YOY walleye and their prey in the late demersal period, walleye did occupy sites within preferred habitat types that had a higher abundance of prey. This suggests that YOY walleye may still be using prey availability as a secondary site selection mechanism in the late demersal period.

Although the prediction that YOY walleye would actively avoid potential predators was not supported by our data, the use of high-cover habitats suggests that young walleye may have been attempting to mitigate the effects of potential predators. Many YOY fishes use vegetated areas of the littoral zone to minimize the risk of predation (Werner et al. 1983; Gotceitas and Colgan 1990). Fishes that face periods of high predation risk often mitigate predation pressure by selecting habitats, such as those with high macrophyte density, that reduce predator efficiency (Savino and Stein 1982; Gotceitas and Colgan 1990). The fact that high prey densities were also present in high-cover areas suggests that YOY walleye may not have suffered any habitat-mediated reductions in growth, unlike most species that use structurally complex habitats to mitigate predation risk (Werner and Gilliam 1984).

While vegetation stands that are too dense can inhibit foraging efficiency (Gotceitas 1990), even the high-cover habitats defined by this study should have been sparse enough to allow foraging while providing some refuge from potential predators. This would put YOY walleye in the beneficial position of reducing predation risk while maximizing growth, the latter being a determinant of survival rate of most fishes (reviewed by Sogard 1997). An early switch to piscivory (Raney and Lachner 1942; Houde and Forney 1970) allows YOY walleye to grow much faster than most other fishes, and it is likely that they are faced with high predation risk for only a short time relative to species that spend years in littoral areas before moving to open water. Wahl (1995) suggested that fast-growing species might have poorly developed antipredatory behaviours because they face a narrow window of predation vulnerability and that these behaviours may be mitigated by habitat selection. This explanation would account for the approximately 3 weeks that young walleye used high-cover habitats. In Big Clear Lake, YOY walleye did not leave the vegetated areas until they were approximately 75 mm in length, which was large enough to substantially reduce the risk of predation once they moved to low-cover habitats.

The rapid growth of YOY walleye in Big Clear Lake continued through the late demersal period, as by early August, YOY walleye had reached approximately 120 mm in length. At that size, the number of potential predators would be greatly reduced. The shallow habitats selected by YOY walleye in the late demersal period could relate to predator

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Table 7. Shoaling behaviour of YOY walleye in the early and late demersal periods as determined by shoal size, the number of YOY walleye shoaling together, and the species associated with walleye in shoals.

Parameter	Early demersal (n = 22)	Late demersal (n = 25)	P
Shoal size (SE)[a]	109.1 (20.9)	25.8 (5.1)	<0.001
Number of YOY walleye in shoal (SE)[a]	4.9 (1.8)	7.2 (1.2)	0.01
Number of YOY-walleye-only shoals	1	9	0.012
Number of times seen shoaling with YOY walleye			
Mimic shiner adults	11	7	0.14
Golden shiner adults	2	10	0.02
Bluntnose minnow adults	3	3	1.0
Bluegill juveniles	1	2	1.0
YOY largemouth bass	1	0	0.47
YOY yellow perch	7	0	0.003
Yellow perch juveniles	1	1	1.0
Walleye yearlings	0	1	1.0

[a]Shoal size and YOY abundance data were $\log_e(x + 1)$ transformed prior to statistical testing, but untransformed means are presented.

avoidance, despite the absence of a significant negative correlation between the abundance of YOY walleye and that of their potential predators. The use of shallow water as a refuge from piscivores has been observed in a number of fishes (Schlosser 1988; Angermeier 1992). One possible explanation for our results is that the PREDIND used in our analysis was too broad and that specific predators influence the distribution of YOY walleye more than others. In particular, the use of shallow habitats by YOY walleye in the late demersal period could be a response to avoid cannibalism by older walleye. This suggestion is supported by the fact that adult walleye were observed on only a single occasion in shallow habitats during the late demersal RVT trials.

Another possible explanation for the inability of our index to detect the importance of predators in the distribution of YOY walleye is that the abundance of some important predator species was affected by diel habitat shifts. In particular, older walleye are more active at night (Ryder 1977), and cannibalism can greatly influence walleye year-class strength (Forney 1976). Other potential predators present in Big Clear Lake are also known to forage nocturnally, including smallmouth bass and brown bullhead (Scott and Crossman 1973). Therefore, habitat-specific predation risk of YOY walleye may exhibit diel variation, and the nonsignificant influence of predators during daylight hours may not be reflective of predation risk during the crepuscular and overnight periods. Species included in the PREYIND are not as likely to be affected by this problem, as they are typically active during the day.

In the months of June and July, some effort was made to assess the nocturnal activity of YOY walleye by returning to observe fish that were observed earlier in the day. These fish were difficult to relocate, but in the two instances where YOY walleye were observed at night, the fish were resting near the substrate and not active. However, adult walleye were often observed moving through shallow areas at night, and these fish were presumably foraging. Thus, future research should address the potential for different patterns in prey and predator abundance between diurnal and nocturnal periods.

Shoaling behaviour

YOY walleye group size decreased significantly between the early and late demersal periods, lending further credence to the suggestion that the selection of highly vegetated habitats during the early demersal period is at least partially due to predator avoidance. Large shoaling groups, like those observed during the early demersal period, help decrease the vulnerability of individuals to predation (Pitcher 1986). The average group size that YOY walleye were associated with during the early demersal period was over 100 individuals, but that number fell to less than 30 individuals by the late demersal period. Similar group size and habitat relationships were noted in comparable size-classes of yellow perch, a close relative of walleye (Eklöv 1997). In that study, small yellow perch (<80 mm) were located in areas of intermediate vegetation density and found in groups of greater than 10 individuals, while large yellow perch (>110 mm) were located in areas with less cover in groups of less than 10 individuals (Eklöv 1997).

YOY walleye tended to shoal with increasingly large fishes as they grew: with YOY yellow perch initially, with adult mimic shiner and adult golden shiner later, and with other YOY walleye by the end of the study. Such size and species assortativeness within fish shoals is expected, as phenotypic homogeneity is an important characteristic of group formation (Krause et al. 1996). By early July, the YOY walleye were larger than YOY yellow perch and adult mimic shiner and were preying on them even as they shoaled together. Walleye are capable of consuming prey half their own length (Campbell 1998), and by shoaling with potential prey during the early demersal period, YOY walleye likely benefit by increasing their predator detection and foraging abilities (Clark and Mangel 1986).

One observation from this study that should be examined in more detail in the future was the apparent stability of YOY walleye shoals in the late demersal period. It appeared that YOY walleye shoals were consistently located in the same general area and that the number of individuals in these shoals was fairly constant (T.C. Pratt, personal observation). Some behavioural work has been conducted on group struc-

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ture and dynamics in yellow perch (Helfman 1984), which were determined to be facultative shoalers. It would be interesting to follow the initial walleye shoals observed here over a few years to determine whether groups remained associated over time, as similar sized groups of yearlings and adults were frequently observed in Big Clear Lake.

Use of the RVT

The specific microhabitat and species association data gathered by this study could not have been collected without using an underwater visual technique, as traditional sampling gear such as seines, gill nets, or electrofishers would have been unable to sample such a diverse fish fauna as effectively or provide the necessary spatial resolution (Sale 1980). Visual methods are not perfect sampling tools (Brock 1982), but given the changes that YOY walleye undergo in their first year, it was decided that visual techniques were most likely to provide answers to the questions posed in this study. Alternative techniques for sampling YOY walleye were tried, including small-mesh gill nets and straight-line underwater visual transects. We chose the RVT because it was found to sample YOY walleye and several of the less abundant species much more effectively than the other techniques (T.C. Pratt, personal observation).

While the RVT has been criticized for overestimating the abundance of evenly distributed species and underestimating the abundance of patchy species (DeMartini and Roberts 1982), the use of the RVT in this study was not specifically to estimate species abundances, but rather to provide relative abundance levels across a number of habitat types. RVT has been compared with more traditional underwater visual methods. Results with the two techniques did not differ significantly in species presence–absence or ranked species abundance, although there were differences between the techniques in the relative abundance of species (Kimmel 1985; Sanderson and Solonsky 1986).

In conclusion, the YOY walleye habitat utilization patterns observed in this study were unexpected, as previous studies had indicated that YOY walleye were habitat generalists. The significant relationship between YOY walleye and their prey during the early demersal period was predicted, although the shift away from this association during the late demersal period was not (Leis and Fox 1996). The results indicate strong selection of heavily vegetated habitats and sites in which prey species were relatively abundant in the early demersal period, followed by a shift to shallower, low-cover habitats. The early habitat selection and shoaling behaviour of YOY walleye suggest that young walleye are influenced by potential predators, and their behaviour (inhabiting areas of high macrophyte density and living in large shoals) is typical of many other prey species living under the threat of predation. It was also apparent, however, that YOY walleye pass this vulnerable period quickly with their rapid growth. Although we were unable to detect negative associations between YOY walleye and their predators, we believe that YOY walleye are sensitive to the risk of predation for a short period during their early life history, and the habitats they select enable them to survive a period of potentially high predation vulnerability.

The shifting patterns of prey and habitat associations detected in our study are important in understanding the early life history of the walleye. The relationships observed here should be the subject of further investigation in an experimental setting in order to further our understanding of the causal factors involved in YOY walleye habitat selection, in particular the role of predation risk on the selection of particular habitats through the demersal period.

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Resolving allegations of oil damage to incubating pink salmon eggs in Prince William Sound

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Abstract: The *Exxon Valdez* Oil Spill Trustee Council concluded that oil caused mortality of pink salmon (*Oncorhynchus gorbuscha*) eggs in Prince William Sound streams. Their conclusion was based primarily on Alaska Department of Fish and Game (ADF&G) studies which reported that mean mortality of embryos in eggs was higher in oiled than non-oiled streams when sampled shortly after spawning completion. However, developing embryos are vulnerable to shock mortality for a period of 20 days after fertilization, and the embryos in eggs from the latest spawners were still in the sensitive period at the time sampling took place. We argue that the original ADF&G analysis should have included sample timing in statistical comparisons of mortality between streams. Analysis of a subset of the ADF&G data showed that sampling shock was a major source of embryo mortality in these samples, and that source of mortality in the original survey would likely have been mistakenly interpreted as an oiling effect. Compensating for sample timing removed all statistical evidence for an oiling effect in the data subset. We conclude that the ADF&G study design confounded the ability to assess for the effect of oil exposure on pink salmon eggs.

Résumé : Le conseil des mandataires du suivi du déversement pétrolier de l'*Exxon Valdez* (*Exxon Valdez* Oil Spill Trustee Council) a conclu que le pétrole est responsable de la mortalité d'oeufs des Saumons roses (*Oncorhynchus gorbuscha*) dans les cours d'eau du détroit du Prince William. Sa conclusion se base principalement sur des études menées par le Ministère de la pêche et de la chasse de l'Alaska (Alaska Department of Fish and Game (ADF&G)) qui indiquent que la mortalité moyenne des embryons dans les oeufs était plus élevée dans les cours d'eau affectés par le pétrole que dans les cours d'eau non-affectés, lors d'un échantillonnage fait peu après la fin de la fraye. Cependant, les embryons en développement sont vulnérables à une mortalité par choc pour une période de 20 jours après la fécondation; or, les embryons dans les oeufs pondus par les poissons qui ont frayé le plus tardivement étaient encore dans leur période vulnérable au moment de l'échantillonnage. Nous croyons donc que l'analyse originale faite par le ADF&G aurait dû inclure la date d'échantillonnage dans les comparaisons statistiques de la mortalité entre les cours d'eau. L'analyse d'un sous-ensemble des données du ADF&G révèle que le choc relié à l'échantillonnage a été une cause majeure de mortalité des embryons dans ces échantillons et qu'une telle mortalité aurait vraisemblablement été interprétée dans l'inventaire original comme étant causée par le pétrole. En tenant compte de la date d'échantillonnage dans le sous-ensemble de données, on enlève toute évidence statistique d'un effet du pétrole. Il apparaît donc, en conclusion, que le plan d'analyse du ADF&G a annihilé toute possibilité de détecter les effets de l'exposition au pétrole sur les oeufs de Saumons roses.

[Traduit par la Rédaction]

Introduction

In March of 1989, the *Exxon Valdez* grounded and spilled 11 million gallons of crude oil into Prince William Sound (PWS). That spring and summer, weathered crude oil washed onshore along approximately 16% of PWS beaches and entered the intertidal zones of an estimated 30 productive pink salmon (*Oncorhynchus gorbuscha*) streams (Fig. 1). Alaska Department of Fish and Game (ADF&G) investigated the effects of oil on pink salmon eggs incubating in these streams after the spawning season in 1989. Higher embryo mortality (17.4% in oiled streams vs. 10.4% in non-oiled) was observed in oiled streams (Craig et al. 1995). Higher mortality was also reported to continue through 1993 (Bue et al. 1996) and reappeared in 1997, well after measurable oil disappeared from the streams. Higher mortality of eggs was reported in oiled streams even above high tide (Bue et al. 1996). Largely from these data, the *Exxon Valdez* Oil Spill Trustee Council (2000) concluded that the reported mortality represented long-term injury to pink salmon in PWS, and reported that patches of oil exposed by winter storms may result in new local episodes of egg mortality.

There were several problems in accepting oil as the cause of higher embryo mortality. First, the mean polynuclear aro-

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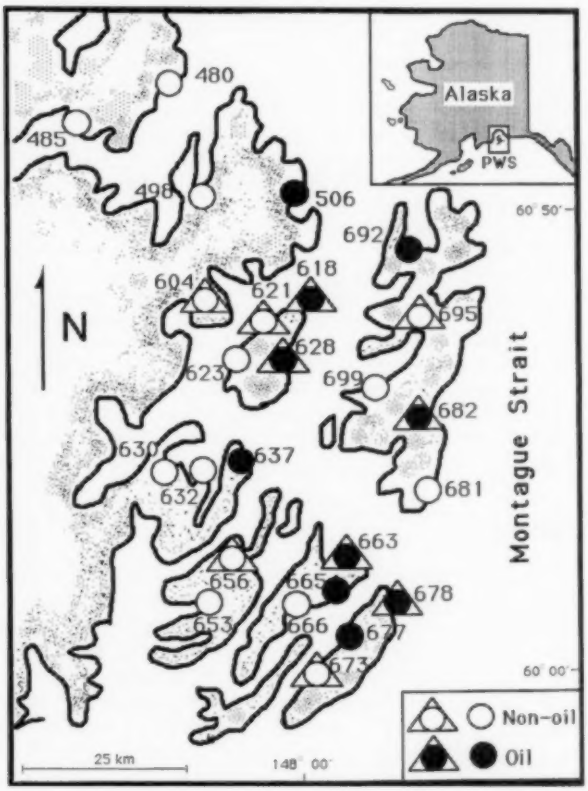
Fig. 1. Prince William Sound (PWS) beach areas where oil washed ashore following the *Exxon Valdez* oil spill. Solid circles indicate the locations of oiled streams and open circles indicate the locations of non-oiled streams. Triangles indicate the 10-stream subset used in the analysis of Alaska Department of Fish and Game data.



matic hydrocarbon (PAH) concentrations in the incubation substrate of oiled streams in 1989 ranged from only 0.5 to 267 ppb (Brannon et al. 1995), well below the 3800 ppb reported as the lethal threshold for pink salmon embryos (Murphy et al. 1999). Contrary to the ADF&G survey data (Bue et al. 1996, 1998), no measurable effects of oil were detected in pink salmon embryo survival in concurrent studies by Brannon et al. (1995), and no differences were observed in percentage of anomalies, time of emergence, or viability, as reported by Marty et al. (1997), in pink salmon embryo laboratory bioassays with the crude oil. Similarly, Moulton (1996) showed no differences in embryo mortality (3.5% in oiled streams vs. 4.9% in non-oiled) in 1989 studies of eggs from returning spawners that were artificially incubated for 2 months in oiled and non-oiled streams until the embryos reached the advanced eyed stage. Moreover, five of the streams reported in Moulton's (1996) study were streams in which ADF&G had reported higher egg mortality (16.1%) a month earlier (Craig et al. 1995). Oil as the source of egg mortality was also questioned when ADF&G reported no differences in over-winter survival of the pre-emergent fry (alevins) among the same streams in 1989 and 1990 (Bue et al. 1996), despite alevins being the life stage most vulnerable to oil toxicity (Moles et al. 1979; Rice et al. 1975).

In reviewing the ADF&G egg survey data, Brannon and Maki (1996) suggested that eggs were sampled too early after spawning and that the mortality reported was actually an artifact of sample timing. Knowledge about the sensitivity of developing salmon embryos to physical disturbance (Smirnov 1954; Jensen and Alderdice 1989; Jensen 1997) is fundamental to understanding the problem. During early development of pink salmon embryos, the delicate vitelline membrane under the chorion envelops the yolk as a barrier between the water and yolk material. Until epidermal elaboration during epiboly replaces the vitelline membrane, the pink salmon embryo is susceptible to membrane rupture from physical shock. At 6–10°C, pink salmon embryos are vulnerable to shock fatalities for a period approximating 20 days (Jensen and Alderdice 1989; Collins et al. 2000) following fertilization. We hypothesized, therefore, that the results of the survey were confounded by egg mortalities from sampling mistaken for the effects of oil.

To test and estimate the effect of sample timing on egg mortality, Collins et al. (2000) conducted a field study in 10 of the 25 streams surveyed by Bue et al. (1996), using the ADF&G hydraulic sampling methodology to assess egg mortality. Two sampling regimes were tested in each stream in 1998: the first (21–27 September) to correspond with the earliest sampling dates employed by ADF&G (Bue et al. 1996) from 1989 to 1997 and the second (16–19 October) to cover the period after embryo development had progressed beyond the point of sensitivity to physical shock. Incubation temperatures at the time of sampling ranged from 6 to10°C. The results of the field tests showed that, when sampled in September, mean mortality for all streams was 63.6% compared with 22.8% when the same streams were sampled in October. The field trials thus demonstrated the major effect that sample timing can have on mortality if hydraulic sampling is undertaken too soon after the end of spawning.

The present paper re-examines the ADF&G egg mortality survey data for potential bias caused by sample timing relative to embryo sensitivity to physical shock. We believe that, if the ADF&G sampling protocol induced bias in embryo-mortality estimates, there is a need to correct this bias, because ADF&G findings are central to the allegation that PWS pink salmon suffered long-term injury from the oil spill. This paper underscores the critical importance of the temporal element that was missing in the original analysis of the egg mortality survey data (Bue et al. 1996).

Data were analyzed to assess the effect of post-spawning incubation time on embryo mortality between oiled and non-oiled streams. The objective of these analyses was to determine whether covariate corrections of sample timing reduced the magnitude of differences in mortality between oiled and non-oiled streams and affected statistical tests on these differences. The hypothesis that differences in sample timing did not affect egg mortality between oiled and non-oiled streams was tested against the alternative that sample timing was responsible for differences in mortality.

Methods

Analysis of the 1989–1997 ADF&G egg survey data subset was

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performed by developing an analysis of covariance (ANCOVA) model to test the major factors that could affect embryo mortality. The variable of interest was sample timing and, hence, the level of embryo development among the latest-spawned eggs of each stream population relative to the end of spawning. Data defining this variable was acquired by integrating sample-timing information from ADF&G records (Craig et al. 1995, 1999) with spawning-ground adult-enumeration summaries (Moulton 1998) from 1989 to 1997. The data set was limited to those streams that were common to both studies (Fig. 1), and included 10 (five oiled and five non-oiled) of the 25 (10 oiled and 15 non-oiled) streams surveyed by Bue et al. (1996). The minimum number of post-spawning days the eggs had incubated prior to sampling was thus calculated as the difference between the estimated date of spawning completion and the date the eggs were sampled (Brannon et al. 1999), and was described as the number of days elapsed ("days") for each population. Table 1 shows statistics on egg mortality and days since spawning completion by stream and year.

Three other components of variability were included in the model. Oil was the original variable reported to affect egg mortality and was included in the model as the type ("type") of stream, representing the oiled or non-oiled classification. The variability among streams ("streams") was also included. A year ("year") effect was included to represent the change in mortality over the 9 years that resulted from differences in the post-spawning time at which sampling was executed from year to year, or from other variables such as a decreasing effect of oiling or variability in natural environmental factors that could have affected embryo mortality.

Based on days ("days") since spawning was completed and the above three factors that could have influenced the level of embryo mortality, the following ANCOVA model was used:

$$(1) \quad p = \mu + \text{type} + \text{stream(type)} + \text{days} + \text{days}^2 + \text{year} + (\text{type by year}) + \text{error}$$

The proportion (p) of dead embryos in each stream was computed as the number of dead eggs divided by the number of eggs and alevins for each year of the ADF&G surveys from 1989 to 1997. The proportion of dead embryos (p) was transformed with the arcsine of the square root to normalize residuals. The mean transformed proportion is symbolized by mu (μ). A test of stream type compares mortalities for oiled and non-oiled streams averaged over all 9 years. Stream was treated as a random nested effect, and stream(type) was used to test type. Since type compares means over all 9 years for oiled and non-oiled streams, it did not provide a very useful test for an effect of oiling, unless an effect of oiling was present in all 9 years. More useful tests, therefore, were the type-by-year interaction and contrasts. The type-by-year interaction tested the overall equality of differences in mortality for each of the 9 years between oiled and non-oiled streams. A type-by-year interaction could result from significant differences in some years and not others. In addition, separate contrasts (t tests that use the error term and degrees of freedom form the ANCOVA model) between oiled and non-oiled streams for each year were tested.

Linear and quadratic covariate effects (days and days^2, respectively) were used to model the number of days since spawning completion and embryo mortality, because exploratory analyses showed that their relationship was curvilinear. A loess plot, a nonparametric smoothing technique (Green and Silverman 1994), was used to examine embryo mortality versus days since spawning completion. The shape of the loess plot showed that a quadratic regression might be useful over the range in days sampled since spawning completion. Covariates were fit after stream and type, because the number of days since spawning completion differed for each stream (Table 1).

Equation 1 was run without and with covariates and was referred to as the uncorrected model and the corrected model, respectively. Effects in models were fit sequentially. Tests were at $\alpha = 0.05$. Data for 1993 were not available for oiled sites and thus 1993 was not included. Error was modeled as normal. Normality of residuals was tested with the Shapiro–Wilk test (Conover 1980). Covariates were fit after type and stream(type), because the length of time since spawning completion differed among streams and years. Interactions with covariates (e.g., day by year, day by type, day by year by type) were nonsignificant and dropped from the model.

Observed and corrected mortalities were compared, to demonstrate how covariates affected differences in mean mortalities between oiled and non-oiled streams. Observed mortalities were simply the means for categories of oiled and non-oiled streams for each year. Corrected mortalities were adjusted by covariate effects of days since spawning completion, and were estimated from least-squares means. Days since spawning completion were averaged for each year for oiled and non-oiled streams.

To demonstrate differences in mean sample times between oiled and non-oiled streams, the intertidal egg mortality data were also grouped separately as annual means by brood year and stream type. For each of the 9 years, mean mortalities were plotted against the mean number of days of post-spawning incubation experienced prior to the time of sampling. The regression was used to characterize the pattern of sampling between oiled and non-oiled streams, and displayed to emphasize the relationship between mean mortality and sample timing in days of post-spawning incubation by type of stream.

Results

The summary of percent mortalities and days of post-spawning incubation (Table 1) shows the wide range that occurred in each of these statistics. Differences between stream types are very apparent. Cursory examination of Table 1 shows that a pattern of higher mortality is associated with fewer post-spawning incubation days accumulated before sampling. The number of very early samples taken (<10 days, for example) is greater among oiled streams than non-oiled streams (13 vs. 2), demonstrating that the trend in higher mortality is associated with less incubation time.

Results for the uncorrected model (without covariates) show evidence that mean mortalities differed for oiled and non-oiled streams in some years (Table 2). The significant type-by-year interactions ($p = 0.048$) indicate that the relationship between oiled and non-oiled streams differed over years (1989–1997). Contrasts were significant for 1991 and 1992 ($p = 0.014$ and $p = 0.034$, respectively, df = 61); in 1991 and 1992 mortalities were higher in oiled streams by 23.7 and 18.8%, respectively (Table 3). All other contrasts were nonsignificant ($p > 0.05$). The significant year effect ($p < 0.001$) shows that mean mortalities, when computed over oiled and non-oiled streams combined, differed among years. Based on results from the Shapiro–Wilk test ($p = 0.546$, $n = 79$), normal error was appropriate.

The use of covariates (corrected model) eliminated significant effects seen for the uncorrected model (Table 2) that could be interpreted as an oiling effect. Once corrected for sample timing, the type-by-year interaction was nonsignificant ($p = 0.180$), indicating that the difference between oiled and non-oiled streams was similar over time. Contrasts for 1991 and 1992 were now also nonsignificant ($p = 0.413$ and $p = 0.098$, respectively, df = 59). The year effect was still significant, showing evidence that environmental factors unrelated

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Table 1. Percent mortality and number of post-spawning incubation days for pink salmon (*Oncorhynchus gorbuscha*) eggs in intertidal areas of five oiled and five non-oiled Prince William Sound streams from 1989 to 1997.

	% mortality									No. of post-spawning incubation days								
Stream No.	1989	1990	1991	1992	1993	1994	1995	1996	1997	1989	1990	1991	1992	1993	1994	1995	1996	1997
Oiled																		
618	18.4	29.5	34.3	66.9	11.4[a]	30.3	—[b]	4.9	45.2	16	8	8	8	19[a]	15	—[b]	25	6
628	17.0	25.3	21.2	29.9	8.2[a]	58.8	11.5	36.1	60.3	10	14	9	7	18[a]	10	16	21	2
663	16.7	12.3	65.6	21.1	24.8[a]	46.0	15.3	17.7	16.7	19	19	2	9	19[a]	17	25	21	17
678	10.3	41.3	57.0	62.7	7.7[a]	34.4	3.5	22.2	28.9	19	9	1	7	20[a]	28	29	33	10
682	17.0	30.9	57.1	30.2	22.9[a]	62.9	25.6	16.8	34.8	23	28	1	12	—[a]	18	28	33	12
Non-oiled																		
604	17.5	36.5	14.6	17.2	12.6	56.3	25.4	12.3	22.6	19	13	20	13	20	10	29	25	14
621	18.7	31.3	36.5	13.8	17.6	71.0	53.8	16.8	32.2	18	15	22	14	23	13	27	27	10
656	18.8	8.6	25.0	52.1	5.4	67.0	20.2	21.9	36.6	18	26	11	10	23	18	25	36	10
673	3.9	3.9	24.8	7.0	14.9	20.5	16.2	5.7	23.0	22	27	1	11	21	27	34	34	15
695	14.8	34.9	15.8	26.9	16.2[a]	48.5	45.5	17.2	26.0	16	13	11	12	14[a]	13	12	29	6

[a]No enumeration data available.
[b]Only 9 eggs were recovered.

Table 2. Degrees of freedom and p values for main effects, covariates, and interactions for uncorrected and corrected models; covariates effects of the number of days since spawning completion were used only in the corrected model.

	Uncorrected		Corrected	
Effect	df	p	df	p
Type	1,2	0.160	1,2	0.160
Stream (type)	2,61	0.570	2,59	0.463
Days	—	—	1,59	<0.001
Days^2	—	—	1,59	0.036
Year	7,61	<0.001	1,59	<0.001
Type by year	7,61	0.048	7,59	0.180

to oil likely changed over the years from 1989 to 1997. Both days and days^2 were significant ($p \leq 0.001$ and $p = 0.036$, respectively) (Table 2) and the coefficients describe a decreasing upward-concave relationship for these data. Based on coefficients for the intercept (0.785), day (–0.0279), and days^2 (0.00359), an estimated 79% mortality occurred at 0 days since spawning completion and 25% at 36 days since spawning completion. Examining the relationship between residuals and predicted values shows that the covariance model fit the data well. Figure 2*a* shows a loess plot of embryo mortality versus days since spawning completion. The general shape of the loess fit is typical of that described by linear and quadratic terms used in the ANCOVA model, with the relationship between embryo mortality and sample timing being negative and concave upward. Figure 2*b* shows the residuals plotted against predicted values for eq. 1. The scatter of residuals is well balanced about the zero line. Had eq. 1 not explained the curvature, residuals would have been unbalanced in numbers and magnitude of deviations from the zero line. Based on results from the Shapiro–Wilk test ($p = 0.126$, $n = 79$), normal error was appropriate.

Covariate effects of days since spawning completion reduced the difference in embryo mortalities between oiled and non-oiled streams for most years. Table 3 shows mortalities and differences between oiled and non-oiled streams. Differences for observed and corrected mortalities are tabulated. Differences (non-oiled *minus* oiled) in mean days since spawning completion are also tabulated. For 7 of the 8 years, the difference in corrected mortalities was less than that for observed mortalities. In 1995, covariates increased differences in corrected mortalities over those for observed mortalities. In all but 1994, oiled streams were sampled earlier on average than non-oiled streams. Differences in mean days since spawning completion ranged from 0.9 to 8.8 days.

The relationship between early sample timing and instances of high mean mortality levels in stream types is most effectively demonstrated when stream types are separated and identified by year in the regression of mean embryo mortality against mean post-spawning incubation time (Fig. 3). Higher mortalities are associated with the earliest sample timing, which occurred more frequently in oiled streams. It diminishes with time, as the later-spawned eggs develop and embryos become less sensitive to shock from sampling. The exception is the abnormally high mortality in 1994, which was related to exceptionally low water levels from very little

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Table 3. Comparison of observed and corrected mortalities.

	Observed mortality			Corrected mortality			Differences	
Year	Oiled	Non-oiled	Δ mortality	Oiled	Non-oiled	Δ mortality	Observed > corrected	Days
1989	0.159	0.147	0.012	0.178	0.178	<0.001	Yes	1.2
1990	0.279	0.230	0.049	0.261	0.242	0.019	Yes	3.2
1991	0.470	0.233	0.237	0.244	0.180	0.064	Yes	8.8
1992	0.422	0.234	0.188	0.293	0.170	0.123	Yes	3.4
1994	0.465	0.526	–0.061	0.489	0.527	–0.038	Yes	–1.4
1995	0.140	0.322	–0.182	0.205	0.425	–0.220	No	0.9
1996	0.195	0.148	0.047	0.299	0.277	0.022	Yes	3.6
1997	0.372	0.281	0.091	0.254	0.208	0.046	Yes	1.6

Note: Observed mortalities are mean mortalities over streams within oiling type. Corrected mortalities are from eq. 1 and were adjusted for sample timing. Differences in means between oiled and non-oiled streams were calculated for both observed and corrected mortalities. Whether or not observed mortalities were greater than corrected mortalities is tabulated. The difference in mean days since spawning completion between oiled and non-oiled streams is given in the final column.

Fig. 2. (*a*) A nonparametric smoothing fit to embryo mortality. (*b*) The residual versus predicted values for the effects adjusted by covariates of days since spawning completion, eq. 1.

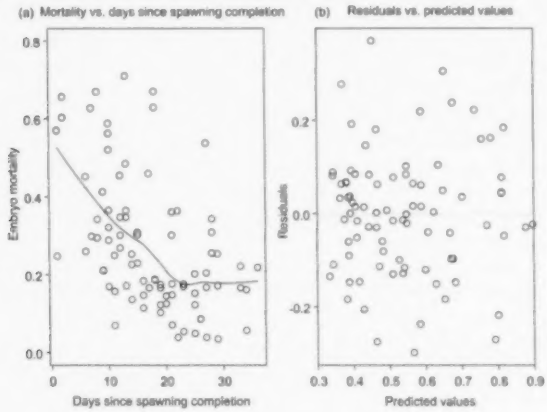
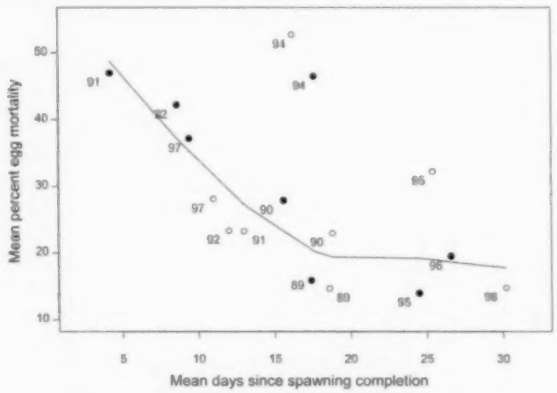


Fig. 3. Mean embryo mortality in intertidal areas of oiled (filled circles) and non-oiled (open circles) streams and mean days of post-spawning incubation at the time of sampling for 10 study streams surveyed in Prince William Sound from 1989 to 1997 (line fit by loess smooth). Mortality data are from Craig et al. (1995, 1999) and Brannon et al. (1999).



precipitation. However, it is also noteworthy that, in 1994, sampling took place within the period of embryo sensitivity but, in this case, it was the non-oiled streams that were sampled earlier and they showed the higher mortality.

Discussion

Covariate corrections of sample timing in the ADF&G data subset show that ADF&G sampling times overlapped the period of embryo vulnerability to physical shock. These analyses suggest that the mortality attributed to oil in the original ADF&G analysis may have been confused with fatalities induced by the sampling procedure. We believe that analysis of the ADF&G survey data subset has shown that sample timing likely induced substantial bias in embryo mortality estimates, owing to sampling being initiated so soon after spawning was completed. Evidence shows that correcting for sample timing was important to avoid misinterpreting the results and attributing embryo mortality to the effects of oiling when assessing for differences between oiled and non-oiled streams. Correcting for sample timing increased the magnitude of the *p* values to nonsignificance for differences that would have been interpreted as an oiling effect. For the uncorrected model, differences in mean mortalities between oiled and non-oiled streams differed significantly among years, as tested by the type-by-year interaction. Significant differences in mortality were eliminated by taking sample timing into account. The same was true for contrasts. Significantly higher mean mortalities were detected at oiled streams in 1991 and 1992 for the uncorrected model. Once corrected for sample timing, contrasts for 1991 and 1992 were nonsignificant. Significant interaction and contrasts would be interpreted as evidence for an effect of oiling, but such evidence disappeared when mortality was corrected for days since spawning completion. This was most apparent for years with the greatest differences in sample timing between oiled and non-oiled streams. With an 8.8-day difference in 1991, the mortality was corrected by 17.3%. When the difference was just over 3 days in 1990, 1992, and 1996, mortality was corrected by 3.0, 6.5, and 2.5%, respectively. If Bue et al. (1996) had included terms to describe the negative relationship between mortality and sample timing in their

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statistical analysis, it is likely they would not have concluded that there was an effect of oiling on embryo mortality, in which case, all studies of oiling effects (Brannon et al. 1995; Bue el al. 1996; Moulton 1996) would have agreed.

Because sample timing overlapped the period of embryo sensitivity to shock among the latest-spawned eggs, the variability in the dates when sampling commenced created a difficulty in maintaining a consistent base of reference on which to compare mortality. Sampling commenced as early as 21 September in 1992 and as late as 10 October in 1995, representing a difference of nearly 3 weeks (Craig et al. 1995, 1999). The number of post-spawning incubation days accumulated before sampling from the first to the last streams sampled ranged from 7 to 22 days over 9 years. The variation in number of post-spawning incubation days that elapsed before sampling ranged from 1 to 36 days. Such large differences in the duration of incubation among developing eggs prior to the time they were sampled grouped the eggs in dissimilar post-spawning incubation periods from year to year and complicated assessment because of differences in sensitivity.

Statistical evidence of a negative relationship between embryo mortality and days since spawning completion for pink salmon in PWS has been shown by two independent sets of data. The first, from a 1998 field study (Collins et al. 2000), demonstrated the negative relationship between days since spawning completion and embryo mortality. The second comprised the significant linear and quadratic terms in the ANCOVA model for data from 1989–1997, which show unambiguous evidence that sample timing should be included in analyses that compare mortalities between oiled and non-oiled streams. This is also apparent from data plots and nonparametric regressions, which clearly show trends over time between mortality and days since spawning completion.

Oiling in itself had no influence on embryo development rate. Although it was demonstrated under laboratory conditions that relatively high oil concentrations caused alevins to emerge prematurely with yolk stores still remaining, altered rates of development were not reported (Marty et al. 1997). In the field studies of oil effects on incubating and emerging fry, emergence timing and fry size were no different between oiled and non-oiled streams, and the index of condition (Bams 1970) indicated that emerging fry were not premature (Brannon et al. 1995).

The influence of temperature on the rate of embryo development was also considered to be a potential influence on embryo mortality. September stream temperatures varied from 6 to 10°C among streams and among years, but the extent of the variation did not exceed a mean of much more than 1°C. In years when August temperatures were higher (e.g., 1991), spawning tended to be later than in years when they were lower, which exaggerated the effect of early sampling. For 1998 data, mortality rates were regressed on water temperature and were shown to be greater in September in streams with higher temperatures, but not in October, demonstrating that higher temperatures enhanced sensitivity (Brannon et al. 1999). Therefore, while variation in temperature could account for some mortality, its primary affect would be to exaggerate mortality from early sample timing.

The results of analyzing the 10-stream subset of the 25 streams sampled by ADF&G suggest that conclusions reached about oiling effects without sample timing being included in the analysis could have been flawed by confusing fatalities from sampling with other effects on embryo mortality. The impact of sample timing is most effectively demonstrated by the 1990, 1991, 1992, and 1997 data. In each case, sampling occurred earlier in oiled streams than non-oiled streams and substantially overlapped the period of sensitivity to physical shock. The analysis of embryo mortalities by Moulton (1996), and of incubation survival by Brannon et al. (1995), avoided embryo sensitivity in eggs by sampling well after embryo vulnerability to physical shock was past. Analysis of the data subset suggests that, if there were an oil effect in ADF&G survey data, it would have been obscured by sample-timing effects.

In conclusion, the analysis of egg mortality between oiled and non-oiled streams from a subset of ADF&G stream survey data was undertaken. The assessment showed that the reported differences in mortality were likely the result of sampling protocol and unequal distribution of sampling effort over time between stream types. In the instances of higher mortality in oiled streams, eggs were sampled too early to avoid shock fatalities and, since oiled streams were more frequently sampled earlier that non-oiled streams, the increased egg mortality was misinterpreted as an oil-related mortality. The effect of shock from sampling on embryo mortality was shown by Collins et al. (2000)—mortality was nearly three times higher in early samples than in later samples taken from the same set of streams. Differences in egg mortality between stream types was not observed by Brannon et al. (1995) and Moulton (1996), because sampling was undertaken well past the period of embryo sensitivity to shock, and timing was not critical. This re-analysis helps to resolve differences in published results on the effect of oiling in PWS streams following the *Exxon Valdez* oil spill. Based on analysis of the 10-stream subset of the ADF&G 25 stream survey, we conclude that sample timing likely explains why eggs in oiled streams were perceived to have had higher mortality. It also explains why mortality was so variable in both oiled and reference streams. It explains why differences in mortality were reported to have continued after oil left the streams, and why differences occurred in those areas upstream of high tide, beyond the physical presence of oil. Induced fatalities from early sample timing also explain why differences in mortality were reported in eggs but were not observed by ADF&G in the later alevin and fry life stages at the onset of emergence.

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Response of the Cache Creek macroinvertebrates during the first 10 years following disturbance by the 1988 Yellowstone wildfires

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Abstract: We evaluated the effects of disturbance on stream benthic macroinvertebrates at the ecological scales of time, stream size, and burn extent in six segments of Cache Creek over the first 10 postfire years. Postfire changes in macroinvertebrate taxa richness, density, and dominant taxa in the burn streams were significantly different from those in the reference stream. Chironomidae and *Baetis* typically comprised 40–60% of the macroinvertebrate assemblages of burned streams but only 15–18% of the assemblage in the reference site. Coefficients of variation for the 10-year period indicated that richness, density, biomass, and *Baetis* abundance were more variable (1.2–3.5 times higher) in the burn streams than in the reference stream and that variability in Chironomidae abundance in burn sites increased with stream size. Fire effects were not attenuated progressively with increasing stream size, probably because the proportion of the catchment burned did not decrease. However, similar-sized streams in which 68–71% of their catchments burned were more severely disturbed than those in which only 39–47% burned. Long-term effects on the macroinvertebrate community were due largely to the loss of terrestrial vegetation and increased runoff, which caused severe alterations in stream channel conditions and large-scale bedload movement.

Résumé : On trouvera ici une évaluation des effets de la perturbation causée par un incendie du bassin versant sur la faune des macroinvertébrés benthiques d'eau courante dans six segments de Cache Creek, aux échelles écologiques du temps, de la taille du cours d'eau et de l'importance de la zone brûlée, au cours des 10 années qui ont suivi l'incendie. La richesse en taxons, la densité et la liste des taxons dominants chez les macroinvertébrés diffèrent significativement dans les cours d'eau affectés par le feu par comparaison avec les cours d'eau témoins. Les Chironomidae et les *Baetis* représentent normalement 40–60% de la communauté de macroinvertébrés dans les cours d'eau affectés mais seulement 15–18% dans les cours d'eau témoins. Les coefficients de variation de la richesse en taxons, de la densité, de la biomasse et de l'abondance des *Baetis* sur la période de 10 ans sont plus élevés par un facteur de 1,2 à 3,5 dans les sites affectés que dans les sites témoins; de plus, la variabilité dans l'abondance des Chironomidae augmente avec la taille des cours d'eau. Les effets du feu ne s'atténuent pas avec la taille du cours d'eau, sans doute parce que la fraction du bassin hydrographique affecté par le feu ne décroît pas. Cependant, des cours d'eau dont 68–71% du bassin versant a été incendié sont plus perturbés que des cours d'eau de taille semblable dont seulement 39–47% du bassin a été affecté. Les effets à long terme sur la communauté des macroinvertébrés sont dus en majeure partie à la perte de la végétation terrestre et au ruissellement accru, qui causent des modifications importantes dans les conditions du lit du cours d'eau et des mouvements de grande envergure des alluvions.

[Traduit par la Rédaction]

Introduction

Many large-scale natural disturbances, including wildfire, have long-term implications for ecosystem structure and function (Picket and White 1985; Foster et al. 1998). For example, recent work suggests that large disturbances produce landscape legacies that can strongly influence ecosystem processes over long periods of time and are important for understanding ecosystem recovery and succession (Foster et al. 1998; Turner et al. 1998). However, studies of the extended temporal responses of ecosystems to natural disturbance are rare, particularly for flowing (lotic) waters, where annual or more frequent upheavals are considered the norm (Resh et al. 1988; Giller et al. 1991; Mackay 1992). In addition, many studies examining the long-term persistence of lotic fauna typically compare two individual surveys completed years apart, with little or no knowledge of what confounding effects may have occurred in the interim (e.g., Albin 1979; Johnson et al. 1994). We are aware of only a few studies, other than our own (e.g., Robinson et al. 1994; Minshall et al. 1995, 1997), that have routinely examined the effects of disturbance by wildfire on stream ecosystems or their components at time scales exceeding a few years (e.g., Roby and Azuma 1995). This general and specific lack of information is a major impediment to fully understanding the effects of wildfire on flowing water environments and their inhabitants. As a consequence, considerably more de-

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tailed descriptive research is needed before meaningful hypotheses can be formulated or accurate predictions made.

In the present study, we address environmental and macroinvertebrate responses in six streams within a single basin (Cache Creek) over the first 10 years (1988–1998) following disturbance by wildfire. This study differs from our earlier work on streams in the same area (Yellowstone National Park) (Robinson et al. 1994; Minshall et al. 1995, 1997) in that it focuses on individual streams longitudinally placed within a single basin and incorporates an additional 5 years of data. This treatment allows us to emphasize fluctuations and variability in a so-called "variance" approach as opposed to "homogenizing" the data by combining sampling sites or times in an effort to simplify the data and detect presumed common patterns (Resh and Rosenberg 1989).

Here, we document changes in Cache Creek over four stream sizes (first to fourth order) during the 10 years following wildfire to determine the extent to which recovery by the lotic macroinvertebrates took place and whether it differed according to stream size. We also wanted to discern whether responses in heavily burned catchments (>60% of total area) differed from those in less severely burned (<50%) catchments. In our earlier study (Minshall et al. 1997), we did not examine the longitudinal responses of individual streams within a single basin, and the results from less completely burned catchments were combined with those of more completely burned ones. Thus, the present analysis is more refined than the earlier one and includes an additional 6 years during which some of the most striking differences regarding stream size occurred. Here, we were concerned with the effects of disturbance of lotic systems at spatial scales of stream size and burn extent within a single catchment and across a temporal scale of 10 years at annual intervals. Our null hypotheses are as follows. (*i*) There are no differences in environmental or macroinvertebrate community attributes between the unburned reference stream and the burned streams, in terms of absolute values, degree of variation, or general pattern of responses. (*ii*) There are no differences in community attributes immediately following the fire or soon after it (presumably the period of immediate or early impact by the disturbance) and in subsequent years (our interest here is in any long-term trends or patterns that were not evident in shorter (e.g., 4-year) data sets). (*iii*) There are no differences in community attributes among streams of different size within the same basin. (*iv*) There are no differences in community attributes between streams whose catchments were moderately burned (i.e., >25 to ≤50%) and those whose catchments burned more extensively (≥60%).

Rarely in the study of large-scale natural disturbances, particularly in remote areas like Yellowstone National Park, can one know unequivocally the conditions prior to the disturbance. In such unplanned "experiments," the investigator must rely on information gathered from a nearby reference site that has not been subjected to the disturbance and an understanding of basic ecological principles (e.g., Frost et al. 1988; Underwood 1992; Wiens and Parker 1995). The use of a reference system for comparison of long-term changes in treatment systems is a widely applied and documented method for unplanned or natural experiments in place of the more preferred pre- and post-disturbance comparisons (e.g., Peterson and Pickett 1995; Barbour et al. 1996). In this study, we selected Amphitheater Creek, in an unburned catchment immediately adjacent to Cache Creek, as our reference condition. The reference stream serves as our best available surrogate for the "before" state of the treatment (burn) streams and also as a "control" for non-fire-related environmental variables, e.g., annual differences in weather or runoff (Underwood 1992).

Materials and methods

Description of study area and sites

Cache Creek is located in the northeast corner of Yellowstone National Park, Wyoming, U.S.A. (Fig. 1). The basin extends east to west and covers an area in excess of 18 000 ha. Human impacts are indiscernible; access is by trail located about 0.5 km or more from the stream, except at stream crossings. The topography is mountainous and extends in elevation from 3300 m in the headwaters to 2100 m at the confluence of Cache Creek with the Lamar River. The Cache Creek catchment burned by crown fire on August 20, 1988, as part of the Clover-Mist Fire (the largest fire complex in Yellowstone in July–August) (Rothermel et al. 1994). The Cache Creek sites were first sampled October 1–3, 1988, and then annually (except in 1996) in early August. Prior to the fire, north-facing slopes were heavily forested with lodgepole pine (*Pinus contorta*) or Englemann spruce (*Picea englemannii*); the drier south-facing slopes were more moderately forested and elsewhere were vegetated by a grass–sagebrush (*Artemisia tridentata*) steppe community. Riparian vegetation was primarily willow (*Salix*) and alder (*Alnus*).

The climate is cool, with mean air temperatures ranging from –11.4°C in January to 10.8°C in July. Mean air temperature of the area consistently remains below freezing until about April, and summer maxima approach 30°C in August. Water temperatures in second- and third-order Cache streams remain at or below 5°C until early June. Mean water temperatures for July are about 10°C and maximum water temperatures of about 20°C occur in August. Minimum water temperature is 0.5°C or less from late autumn through early spring. Mean annual precipitation is 56.3 cm (Dirks and Martner 1982) with a winter peak from snowfall. In Yellowstone, 75–85% of the precipitation occurs as snow or rainfall on snowpack (Despain 1987). The summer of 1988 was the driest recorded since 1886, with precipitation in June, July, and August of 20, 79, and 10% of average, respectively (Turner et al. 1997).

We concentrated our efforts on six stream sites that ranged in size from first through fourth order (Table 1). An adjacent, essentially unburned (2% burned) watershed (Amphitheater Creek) served as a reference. However, because of funding constraints, it was not examined in 1993–1995 or 1996. Few catchments having streams larger than fourth order were affected directly by the fires in the Greater Yellowstone Ecosystem. In the Cache Creek catchment, one site of each size was heavily burned (roughly 60–70% of the upstream catchment burned, 44–53% of which was forested); the second- and third-order heavily burned sites were paired with more moderately burned sites (Lower Cache 2 and South Fork Cache, respectively) (39–47% of the upstream catchment burned, <30% of which was forested). Discharge values reflected stream order and catchment area (Table 1). Hydraulic slopes were steeper (≥9%) in the headwaters (first and second order) than in the downstream sites (1–3%). The gradient of the reference site was intermediate between the two sets of burn streams. Chemically, the stream water was moderately high in ion content (Table 1). Specific conductance, an indicator of total dissolved solids, was highest in the Cache headwater tributaries, intermediate in South Fork, and lowest in the mainstem. The reference stream was slightly

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Fig. 1. Location of Cache Creek (burned) and Amphitheater (reference) sites. The two upstream Cache tributaries (UC1, UC2) and the two mainstream sites (C3, C4) were the most severely burned. The two downstream Cache Creek tributaries (SC3, LC2) were less heavily burned.

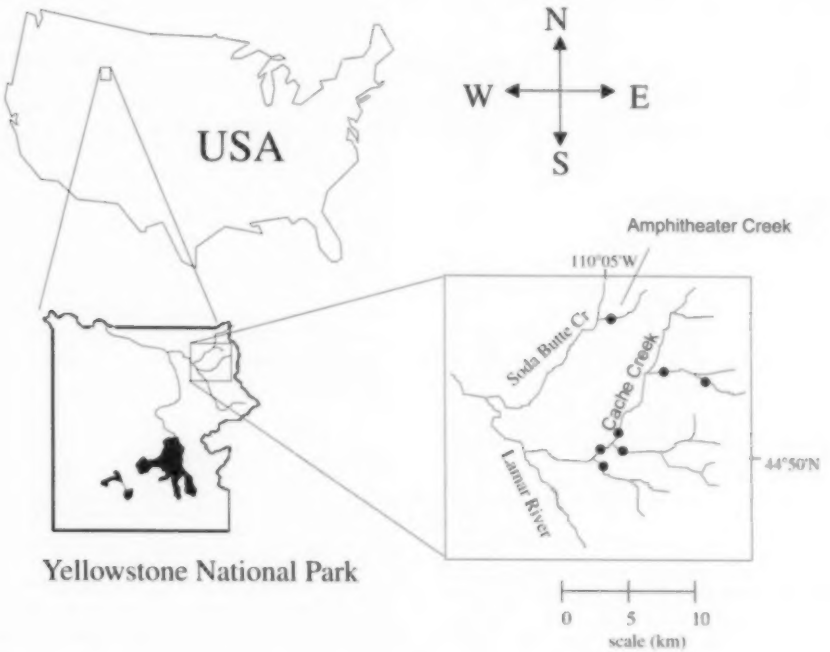

Table 1. Physical and chemical descriptions of the Cache Creek study sites.

Site name and order	Total burn (%)	Canopy burn (%)	Watershed area (ha)	Discharge ($L \cdot s^{-1}$)	Slope (%)	pH	Total alkalinity (mg $CaCO_3 \cdot L^{-1}$)	Conductance ($\mu S \cdot cm^{-1}$ at 20°C)
Upper Cache 1	64	49	138	4 (2)	13 (2)	8.0 (0.2)	62 (7)	125 (13)
Upper Cache 2	71	53	377	12 (7)	10 (1)	8.2 (0.1)	66 (12)	109 (21)
Cache 3	68	53	12 052	475 (191)	2 (1)	8.4 (0.3)	38 (5)	68 (8)
Cache 4	59	44	17 995	668 (243)	1 (1)	8.3 (0.5)	43 (11)	68 (15)
Lower Cache 2	47	23	781	12 (3)	9 (1)	8.3 (0.2)	90 (8)	169 (17)
South Fork Cache 3	39	28	5 781	195 (118)	3 (1)	8.3 (0.5)	43 (5)	81 (14)
Amphitheater 2	2	0	2 535	147 (41)	5 (1)	8.2 (0.1)	34 (6)	58 (7)

Note: Discharge, slope, and water chemistry values were collected under baseflow conditions. Data are presented as means (±1 SD) calculated over the duration of the study.

lower in total dissolved solids than the burn sites. Calcium is the predominant cation and carbonate is the predominant anion.

Total annual precipitation during the study period at recording stations nearest the headwaters of Cache Creek was lowest just preceding and coincident with the fire (1987, 1988) and in 1994; this was interspersed by an extended period (1989–1993, 1995) of intermediate precipitation (Fig. 2). Average annual discharge, as recorded in adjacent unburned Soda Butte Creek, closely followed precipitation, with highest values (approximately two times the lowest) occurring near the end of the 10-year period (1996, 1997) (Fig. 2). The hydrograph is dominated by snowmelt runoff (May–July) peaking in early June, with flows at or near baseflow from August through March.

Field and laboratory procedures

We focused on factors that would provide a foundation for study of the mid- and long-term responses of stream ecosystems to wildfire. Consequently, most of our methods are routine in stream ecology and are described in detail elsewhere (Davis et al. 1999; or see references in Minshall et al. 1997). Details for relatively nonroutine procedures are given below.

Five permanent transects, each including one or more associated fixed-point photograph locations, were established at approximately 50-m intervals at each 200-m-long study site to measure channel morphology and riparian conditions over time. Each transect was a line extending across the stream (approximately perpendicular to the flow path) being marked at each end by metal pins, between which a meter tape was stretched at the time of measurement. The lower and upper transects (Nos. 1 and 5) delineated the downstream and upstream boundaries of each study site. The location of the initial transect at each site was predetermined from topographic maps and aerial reconnaissance. Channel cross-sectional profiles (bankfull width, depths) were measured at each transect. The degree of annual channel alteration also was determined separately by two observers (G.W.M., T.V.R.) based on photographic records for each site. For this, the change in conditions from one year to

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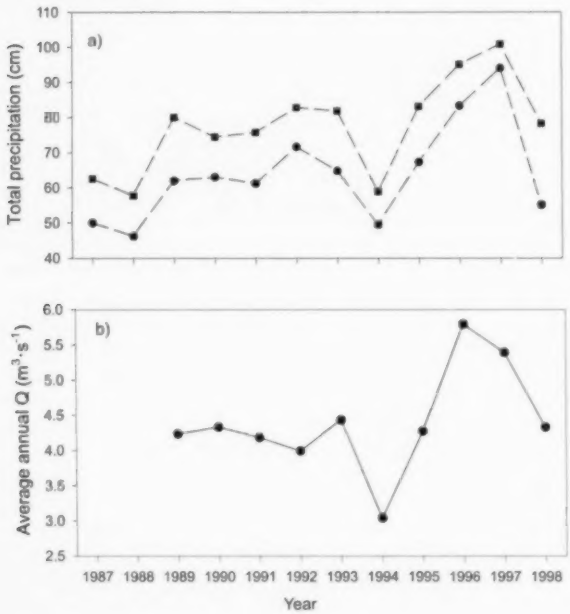
Fig. 2. (*a*) Total annual precipitation for Northeast Entrance (circles) and Parker Peak (squares) and (*b*) average annual discharge for Soda Butte Creek. These locations are the recording stations nearest Cache Creek.



the next was subjectively scored from zero (no change) to five based on extent of channel widening, streambed erosion and deposition, thalweg shift, and woody debris accumulation and removal. Total annual scores were standardized to eliminate differences in the number of photopoints among transects and sites. These standardized scores (as percent change) for the two observers where then averaged.

Most of the routine sampling occurred during baseflow. Discharge was calculated at one transect at each site at the time of sampling (Bovee and Milhous 1978). Substratum size (*a*-axis) was determined for 100 random observations distributed throughout the 200-m reach (Leopold 1970; Platts et al. 1983). Chemical measures recorded when sampling at each site included specific conductance (Orion model 250) and pH (Orion model 125). In addition, water samples were collected, stored on ice, and analyzed in the laboratory for total alkalinity and total hardness using standard methods (American Public Health Association 1989). Benthic macroinvertebrates were handpicked from each sample (Surber sampler, 250-μm mesh, $n = 5$ per site per sample date) and identified to the lowest taxonomic unit feasible (usually genus) using a dissecting microscope at 10× magnification. Taxonomic designations for all years were standardized to those in use in our laboratory in 1998; this may cause some differences between metric values published previously and those reported here. After identification, the macroinvertebrates were dried at 50°C and weighed on a Cahn electrobalance to determine biomass. Associated plant matter was dried, as described above, and combusted to a constant weight at 550°C to determine standing stock of benthic organic matter in the stream.

Statistical procedures

Statistical analysis of time series patterns among sites was limited by the high degree of heteroscedasticity in the data, the absence of data for 1996 for all sites, and the absence of data for the reference stream in three additional years. However, analyses were run for the years 1988–1992 and 1997 combined (since these were the years in which we had observations for all five locations) using a mixed-model approach (Littell et al. 1996). Levene's test (Minitab, Inc. 1998) was used to establish heteroscedasticity before running the mixed model and later to test for equality of variances between burn and reference sites of the combined 10-year coefficients of variation. In the mixed-model approach, year and location were treated as fixed and transect as random, and the variance covariance structure was then modeled using Akaike's information criterion and Schwarz's Bayesian criterion to choose the best covariance structure (Littell et al. 1996). Next, contrasting the unburned Amphitheater site with the average of the four burned sites tested for the main effect of location. Also, the burned sites only were examined, using data from all years studied, in an ordinal approach to quantify differences in response among longitudinally linked locations. The mixed-model analysis was performed using SAS (SAS Institute Inc. 1998). In addition, the correlation between channel change and richness was tested using the Pearson product-moment coefficient, and the relationships between the differentially burned pairs of streams were quantified statistically using a Mann–Whitney test (Zar 1999).

Results

We examined disturbance of natural stream ecosystems by wildfire at the scales of time (years), stream size, and burn extent. We present the temporal and size aspects for the four heavily burned sites first, followed by a comparison of results from the two paired sets of second- and third-order differentially burned sites (see Table 1: Upper Cache 2 (UC2) versus Lower Cache 2 (LC2) and Cache 3 (C3) versus South Fork Cache 3 (SC3)).

Temporal and longitudinal patterns at severely burned sites

Benthic habitat

Stream channel cross-sectional morphology and location of the thalweg at the individual transects changed dramatically from year to year in all burn streams. However, mean values among years at each site were more consistent. Temporal changes in mean active channel width differed between small (first and second order) and large (third and fourth order) burn sites (Fig. 3). The small streams increased sharply in width in 1989 due to braiding and then narrowed again in 1991–1992 as new flow paths were established by downcutting and bank slumping. Upper Cache 1 (UC1) showed a wide range of responses in 1992 as indicated by the large standard deviation), but the net (mean) effect was for width to increase slightly more than in 1989. Following 1992, mean widths for UC1 were similar to or less than those just after the fire. UC2 responded differently than UC1 in that mean width decreased (and the standard deviation was less) in 1992 and then increased in 1993 (with a moderately high standard deviation) in response to changes in deposition and erosion. In contrast, active channel width was relatively stable or decreased slightly at the larger sites from 1988 through 1995 and then increased substantially to exceed the 1988 values with the onset of record-high runoffs near the end of the study period. Mean width decreased progressively after the fire at the reference site, in a manner similar to Cache 4 (C4), until 1992, but mean channel width remained narrower

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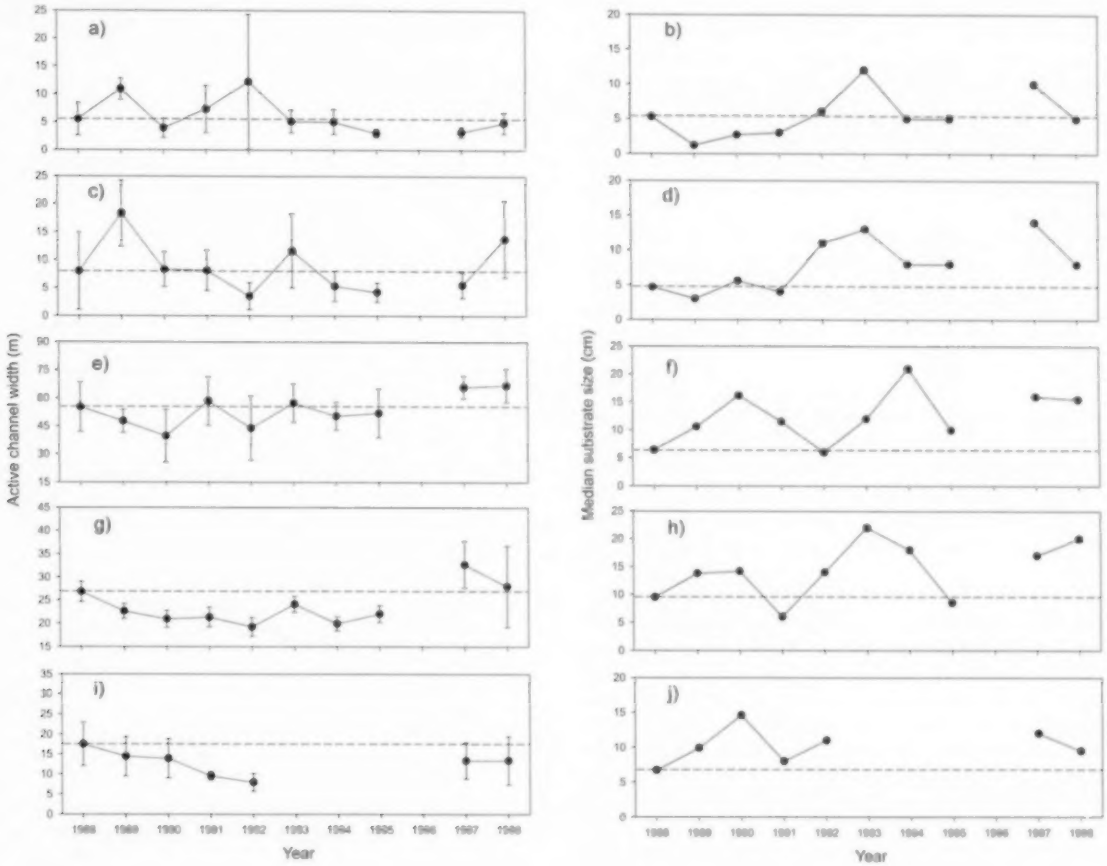
Fig. 3. Active channel width and median substrate size for the reference and four severely burned Cache Creek sites: (*a* and *b*) UC1; (*c* and *d*) UC2; (*e* and *f*) C3; (*g* and *h*) C4; (*i* and *j*) Amphitheater. The broken line is a visual reference for the value just after the fire. Error bars are ±1 SD.



than in 1988 instead of increasing in the final 2 years, as occurred at C4.

Median substrate particle size at UC1 remained at or below the initial value in all but two years (Fig. 3). At the remaining sites, median sizes during the 10 years generally were equal to or greater than those at the beginning. The temporal pattern in median substratum size in the reference stream was similar to that at C4 in those years for which comparable data are available. Although it was unfortunate that the reference stream could not be sampled more frequently over the study period, we believe that conditions remained relatively constant during the period when sampling occurred at the fire-disturbed sites. For example, the three missing years (1993–1995) of data for Amphitheater Creek were from a period of normal to below-normal precipitation and discharge (Fig. 2); thus, little change in stream channel conditions would be expected. In addition, the stream drains an undisturbed forested catchment, and its channel remained stable over the five preceding annual sampling dates; thus, it is likely that instream conditions also remained relatively stable from 1993 through 1995. However, the same cannot be assumed for 1996 because precipitation and discharge were above normal and marked stream channel and biotic changes were observed the following year (1997) when all sites, including Amphitheater, were sampled.

Active channel width, cross-sectional area (results not shown), and substratum particle size did not fully reflect the observed disturbance of the stream channel in any given year. Although the available precipitation and discharge records (Fig. 2) describe the general background conditions affecting the study sites, they do not include a major additional source of disturbance to the burned catchments resulting from changes in the timing, amount, and rate of runoff due to reduced evapotranspiration, retention, and infiltration of precipitation. These "unrecorded" disturbances were especially important in the early years (1989–1993) following fire. Therefore, we used an index of channel change based on the photopoint records (Fig. 4) to better indicate the extent of physical disturbance each year (primarily associated with spring runoff). These results indicated a high degree of channel change in 1989 in the first- through third-order burn segments resulting from high snowmelt runoff in the spring following fire. Runoff at these sites apparently was less intense in 1990 because the extent of channel change was rated low, even though background precipitation and discharge were similar to those in 1989 (Fig. 2); the remaining period of near-normal

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Fig. 4. Index of channel change derived from visual evaluation of annually repeated photographs from fixed points: (*a*) UC1; (*b*) UC2; (*c*) C3; (*d*) C4; (*e*) Amphitheater.

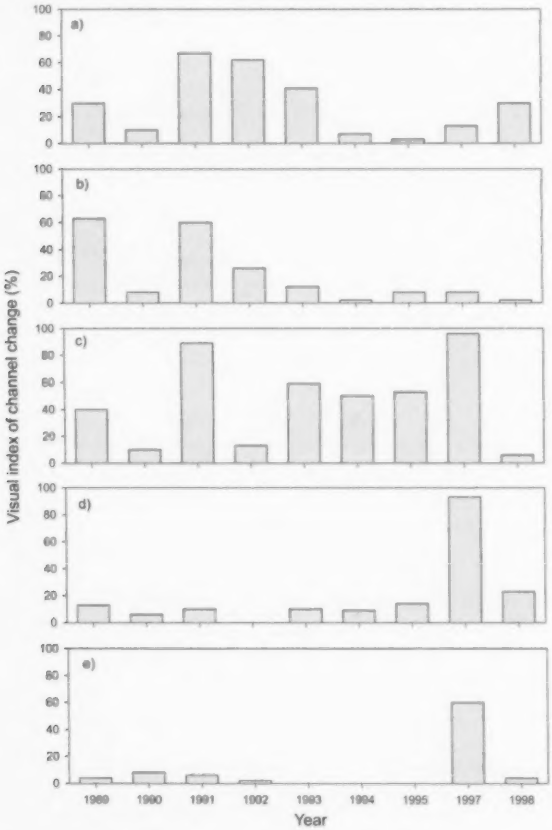
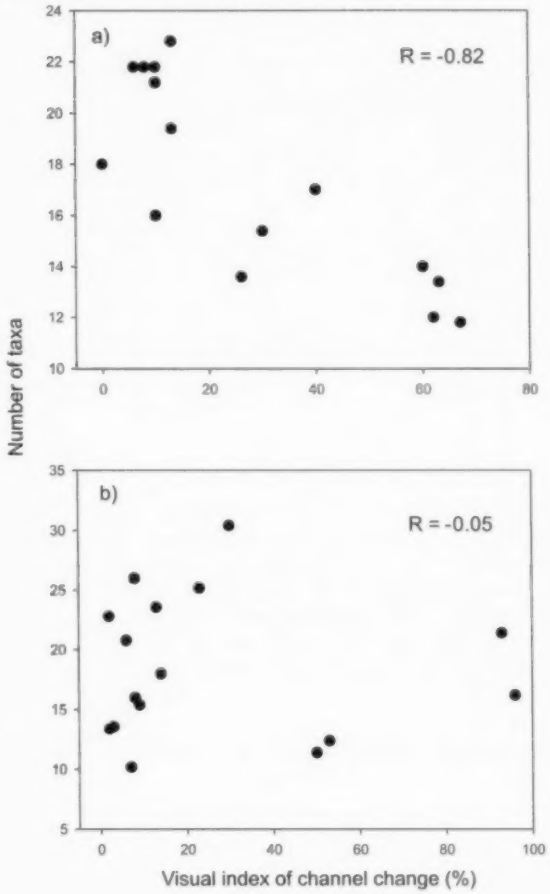


Fig. 5. Macroinvertebrate taxa richness as a function of stream channel disturbance for the reference and four severely burned Cache Creek sites: (*a*) 1989–1992; (*b*) 1994, 1995, 1997, 1998.



hydrologic conditions (1991–1993) was marked by one or more substantial channel alterations at these three sites. In the latter half of the study, the channels at UC1 and UC2 changed less than in the initial postfire years. C3 was the most disturbed of the four sites, with index values ≥40% change in six of the nine years scored. C4 consistently had the lowest index values of the burn streams (≤20% change) until 1997 and 1998. Only C3 and C4 were altered substantially (higher than any previous time or other sites) by the above-average precipitation and runoff of 1997. Changes in the reference stream were similar in pattern to those at C4 but less intense (except 1990). Comparison of Figs. 2 and 4 indicates that the index of channel change for C4 and Amphitheater was mainly associated with high precipitation and runoff (1977). In contrast, the remaining burn sites (C1–C3) changed sporadically throughout the 10-year period, irrespective of precipitation and discharge.

Macroinvertebrate community responses

Mean macroinvertebrate richness at UC1 was consistently lower (10.2–16.0) and that at UC2 generally higher (13.0 – 21.8, except 11.4 in 1993) than the initial 1988 value (17.0 at UC1, 11.4 at UC2), except for much higher values in years 9 (23.6 at UC1, 26.0 at UC2) and 10 (30.4 at UC1, 22.6 at UC2). In contrast, the two downstream burn streams varied between being similar to the initial value, during the early part of the study, and being markedly lower in the period 1993–1995 (range of low means: 10.2–12.4 at C3, 14.6–15.4 at C4). But by 1991, values at both sites had returned to values comparable with those in 1988. In contrast, richness for the reference stream was higher than the initial assessment (14.4) for all subsequent years (20.0–22.8), including the last (28.0). The apparent increases in richness in three of the four burn streams in 1998 also occurred in the reference stream and thus cannot be attributed solely to wildfire.

When macroinvertebrate taxa richness values for all four burn sites were combined and compared with the extent of channel change (Fig. 5), there was a negative correlation (Pearson correlation $r^2 = -0.82$) for the first half of the 10-year period. A comparable, but weaker ($r^2 = -0.62$), relationship (not shown) occurred between richness and change (from the previous year) in the active channel width. However, Sorenson's qualitative similarity index indicates that a basic suite of taxa (around 50% of the initial 1988 community) generally were present at a site, although differing in

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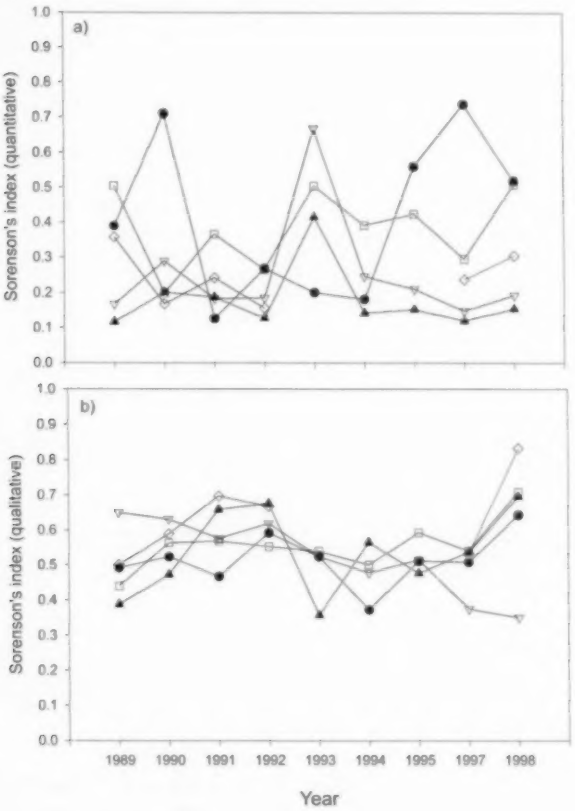
Fig. 6. Sorenson's similarity values relative to 1988 for the reference and four severely burned Cache Creek sites: circles, UC1; squares, UC2; solid triangles, C3; open triangles, C4; diamonds, Amphitheater. (*a*) The quantitative scores include taxa densities, whereas (*b*) the qualitative scores are based solely on presence–absence.



their abundance (Fig. 6). Sorenson's quantitative index (Fig. 6*a*) showed no discernable temporal pattern among sites. We attribute this to the high year-to-year variability in the occurrence of various taxa that strongly affects this index. Sorenson qualitative similarity values in the reference stream were as high or higher than in the burn streams in all years measured (Fig. 6*b*). Values in the reference stream never fell below 50%, and the 1998 level (83%) was 12–48% higher than in any of the burn streams. UC1 and UC2 values behaved somewhat comparably with each other. UC1 levels remained relatively constant around 50% with notable upward excursions in 1992 and 1998. UC2 values, after increasing to above 50% in 1990, stabilized around 55%, except for the pronounced increase in 1998. At C3, similarity showed the greatest temporal variability of all sites. It increased from 39% in 1989 to 68% in 1992 and then plunged to 36% in 1993 before gradually building to 70% in 1998. The pattern of similarity was also unique at C4, where values gradually declined over time from 65 to 35%. C4 similarity was the highest of all sites in 1989 and the lowest in 1998. All other sites had their highest similarity values of the study in 1998 and showed marked increases over those in 1997.

Midges (Diptera: Chironomidae) and *Baetis* (Ephemeroptera: Baetidae) collectively comprised a much larger proportion of the benthic fauna in the burn streams than in the reference stream. The temporal pattern in Chironomidae abundance (Fig. 7) was similar at C3 and C4, decreasing progressively from 1988 to 1992, increasing in 1993, and then declining again to lows that were maintained through year 10. *Baetis* abundances in the burn streams generally were the opposite of those of chironomids. In the reference stream, Chironomidae and *Baetis* values were near 10% or less of the assemblage throughout the 10 years. In 1988, the percent dominant taxon (Plafkin et al. 1989) ranged from 51 to 70% in the four burn streams but was considerably lower in Amphitheater at 28%. Within the burn streams, percent dominance consistently reflected the abundance of either Chironomidae or *Baetis* (Fig. 7). Together, these two taxa represented >85% of the benthic community in UC1 in 1990 and 1995. Chironomidae and *Baetis* combined typically represented approximately 40-60% of the community at the other three burned sites but only 15–18% of the community in Amphitheater. The dominant taxa in Amphitheater varied among years but commonly included *Rhithrogena* (Ephemeroptera: Heptageniidae) and early-instar Plecoptera in the family Nemouridae.

To better quantify the differences in macroinvertebrate assemblage measures among sites, we examined their coefficients of variation for the 10-year period (Fig. 8). Taxa richness (except for C4), density, and *Baetis* abundance were more variable in the burned streams than in the reference stream. In addition, richness coefficients of variation for the burn streams decreased progressively with increasing stream size. Also, variability in Chironomidae abundance within the burn sites increased with increasing stream size, but that of *Baetis* remained relatively constant. Levene's test was used to examine for equality of variances, with the four burn streams treated as one group and the reference site forming another. The results indicate that over the study period, mean values of density (Levene's statistic (LS) = 6.45, $p < 0.0001$), biomass (LS = 5.00, $p < 0.0001$), richness (LS = 1.56, $p = 0.021$), percent Chironomidae (LS = 4.27, $p < 0.0001$), and percent *Baetis* (LS = 3.71, $p < 0.0001$) were all significantly more variable in the burned streams than in Amphitheater.

Using a mixed-model approach (SAS Institute Inc. 1998) to examine the variance–covariance structure, we found that richness (F =12, $p < 0.0001$; $F = 30.2$, $p < 0.0001$), density ($F = 4.27$, $p = 0.0117$; $F = 11.52$, $p < 0.0001$), percent Chironomidae ($F = 94.1$, $p < 0.0001$; $F = 79.1$, $p < 0.0001$), percent *Baetis* ($F = 28.4$, $p < 0.0001$; $F = 83.7$, $p < 0.0001$), and biomass ($F = 1.59$, $p = 0.21$; $F = 11.33$, $p < 0.0001$) varied significantly among sites (except biomass) and over time (respectively for the F and p values, using data only for 1988–1992 and 1997 to allow Amphitheater to be included). There also was a significant ($p \leq 0.0005$) interaction between sites and time for each of these variables, indicating that the differences among sites were not constant over time. This supports the observation that the streams showed something of an individualistic response, in that the major fluctuations did not occur in the same years for all sites. Comparison of the mean value for all four burned sites against Amphi-

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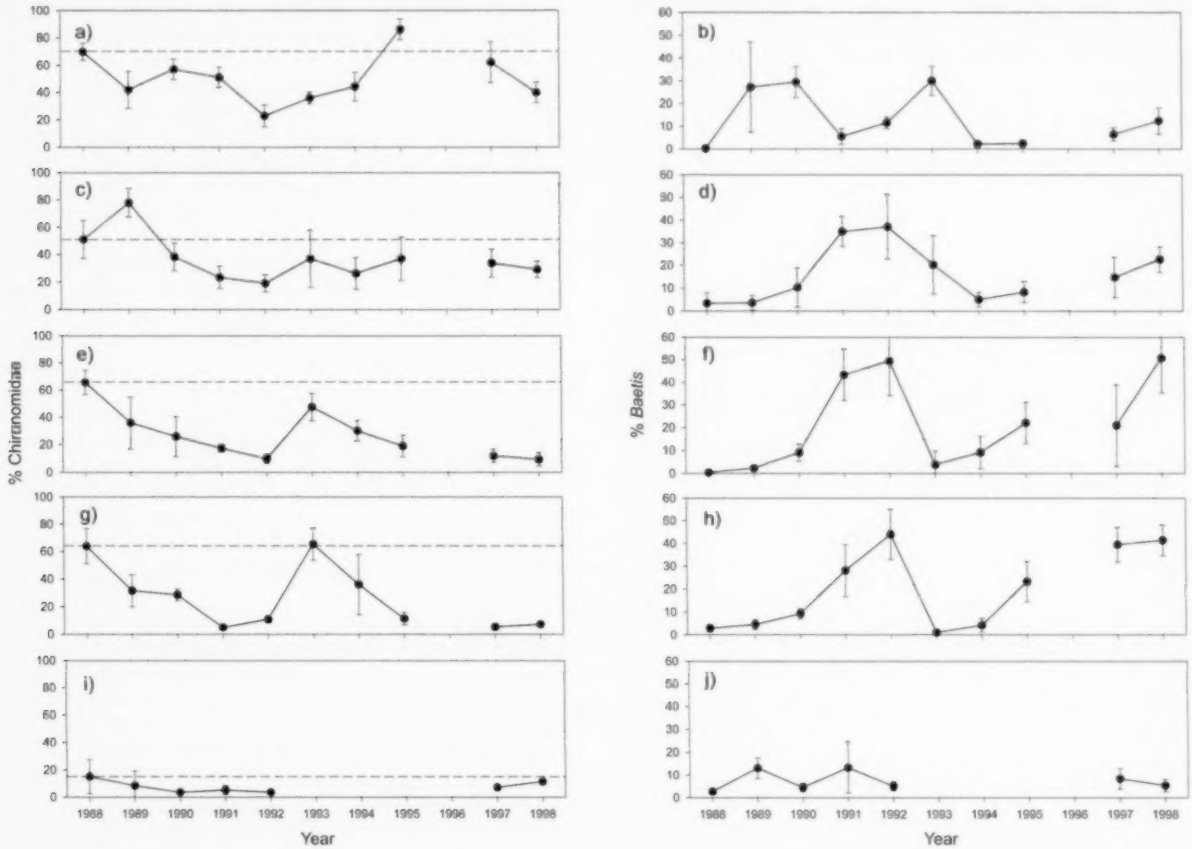
Fig. 7. Relative abundance of Chironomidae and *Baetis* for the reference and four severely burned Cache Creek sites: (*a* and *b*) UC1; (*c* and *d*) UC2; (*e* and *f*) C3; (*g* and *h*) C4; (*i* and *j*) Amphitheater. Error bars are ±1 SD.



theater also showed significant differences for richness (F = 14.1, p = 0.0012), density (F = 9.5, p = 0.058), Chironomidae (F = 197.6, p < 0.0001), and *Baetis* (F = 69.8, p < 0.0001). The lack of significant differences for biomass (F = 2.01, p = 0.17) suggests the possibility of a compensatory response to disturbance. Further statistical analysis was conducted on the four burned sites only using the data from all 10 years studied. These results showed that there were significant differences among sites for richness, Chironomidae, and *Baetis* but not for density and biomass and that grouping the sites into upstream (UC1, UC2) and downstream (C3, C4) often gave a stronger contrast than when the sites were examined individually (Table 2). These results are consistent with the differential response observed for upstream versus downstream habitat conditions.

Response of macroinvertebrates in severely versus moderately burned catchments

Macroinvertebrate total density, total biomass (results not given), and taxa richness were lower in the streams having >60% of their total catchments burned (UC2, UC3) than in those with <50% (LC2, SC3) (Fig. 9). The stream channels of the less severely burned catchments also were observed from annual photographs to be less disturbed in the 10-year aftermath of the fire than were the channels of the more severely burned sites. Median total densities in LC2 and SC3 were twice those in UC2 and C3, respectively. In addition, the median number of taxa was 9–12 times higher in the less heavily burned catchments (Fig. 9) than in the more severely burned ones, but median values were similar within treatments (i.e., UC2 = C3, LC2 = SC3). A Mann–Whitney test of the median values showed that the differences in richness were significant for both the second- (W = 125.5, p = 0.004) and third-order streams (W = 146.0, p = 0.002) but that the differences in density were significant only for the third-order streams (W = 122.0, p = 0.010).

Discussion

Responses of the macroinvertebrate community during the first 10 years

We expected richness and total density of benthic invertebrates to decrease sharply following fire and then recover to prefire levels within the first 5 or so years (Minshall et al. 1989; Minshall and Brock 1991). Instead, both ranged above and below conditions found immediately after the fire throughout the 10-year period (with mean density showing much greater fluctuations than richness), indicating that an equilibrium (such as seen in the reference stream) had not been

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Fig. 8. Mean coefficients of variation for key macroinvertebrate assemblage metrics at the reference and four severely burned Cache Creek sites, excluding an outlier for density at UC1 in 1995 (256×10^3) and another for biomass at C3 in 1992 (324×10^3). (*a*) Taxa richness; (*b*) Chironomidae relative abundance; (*c*) density; (*d*) *Baetis* relative abundance; (*e*) biomass. Note that the scale of the *y*-axis varies from panel to panel.

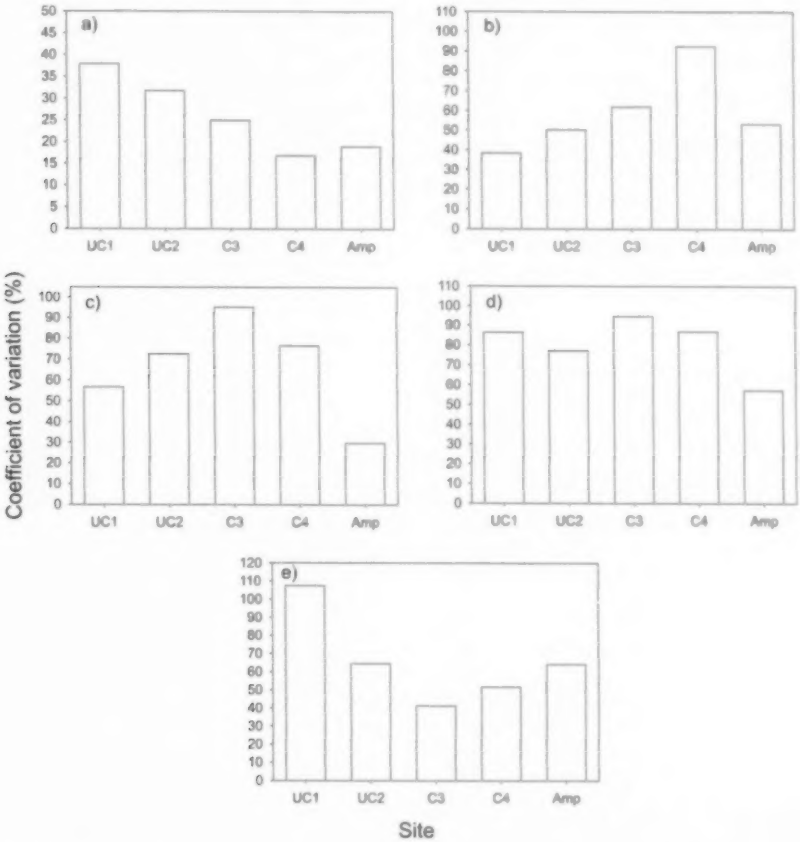

Table 2. *F* values and probabilities resulting from the statistical analysis of taxonomic richness, dominant taxa (Chironomidae, *Baetis*) abundance, total density, and total biomass for the four severely burned stream sites (C1–C4) and all years studied.

	Richness		Chironomidae		*Baetis*		Density		Biomass	
Location	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
C1 vs. C2	3.17	0.0938	20	0.0004	1.59	0.2258	3.84	0.0676	0.0	0.9552
C2 vs. C3	2.47	0.1356	42.01	<0.0001	26.37	<0.0001	1.42	0.2501	0.0	0.9749
C3 vs. C4	19.12	0.0005	10.71	0.0048	34.17	<0.0001	1.84	0.1942	1.44	0.2471
C1 and C2 vs. C3 and C4	42.98	<0.0001	100.11	<0.0001	16.1	0.001	1.57	0.2276	0.58	0.4561

reached. In contrast, richness and density in the reference stream remained relatively constant throughout the 10 years, except for increases in richness in the second and last years of the study. The increase in richness in Amphitheater Creek in the first year following the fire (from 13.6 to 20.4) could have been the result of recovery from drought conditions in 1987 and 1988; such recovery may have been suppressed in the fire-disturbed streams. The increase in 1998 occurred in the burn streams also and cannot be attributed to the effects of fire.

In addition to the marked temporal variability in richness and total density in the burn streams, there were substantial alterations in the composition of the macroinvertebrate assemblage. For example, both Chironomidae and *Baetis* constituted relatively small proportions of the total density (≤15%) at the reference site in all years, whereas they frequently attained levels ≥25% in the burn streams. *Baetis* never exceeded 10% of the fauna in the reference stream during the 10-year period, but it comprised 30–50% of the total numbers at various times in the burn streams. Both taxa are considered by some stream ecologists (e.g., Anderson 1992) to be disturbance adapted, and their highest relative abundance largely coincided with periods of channel disturbance.

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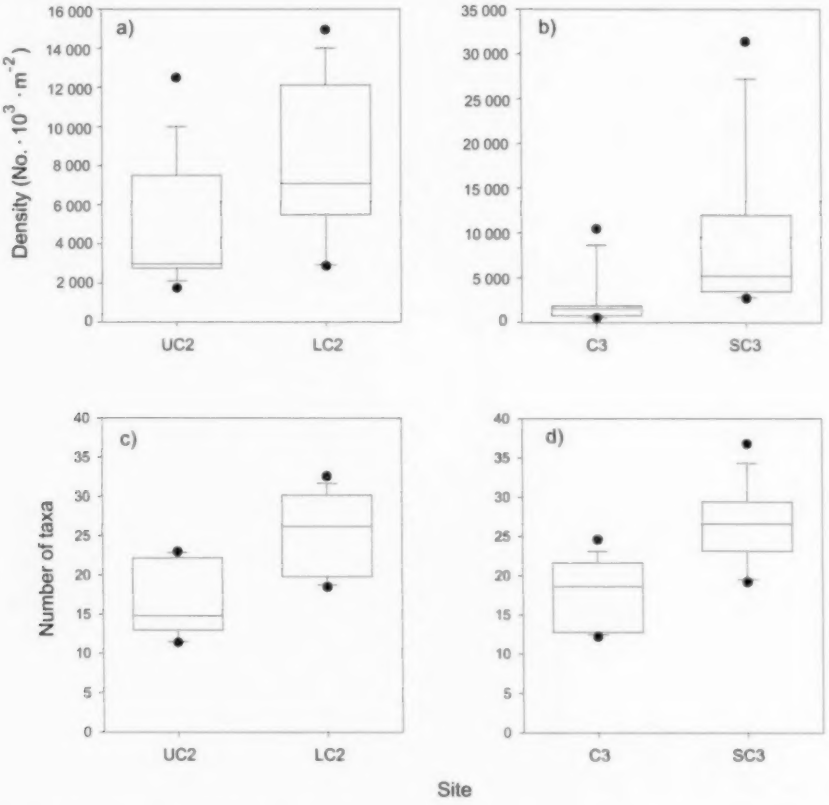
Fig. 9. Box plots comparing macroinvertebrate (*a* and *b*) density and (*c* and *d*) richness from heavily burned tributaries (UC2, C3) and their less-impacted counterparts (LC2, SC3, respectively). Each box plot shows the median, standard deviation, and 90% confidence limits; circles are outliers.



The impact of wildfire on the Cache Creek zoobenthos extended well beyond the fire itself, with some changes evident even after 10 years. This prolonged effect was due largely to the loss of terrestrial vegetation and increased runoff, which in turn caused severe alterations in stream channels and large-scale bedload movement. This directly influenced the temporal variation within and among sites in macroinvertebrate density and composition. These changes were exacerbated in the larger third- and fourth-order sites by increased runoff resulting from above-average precipitation in the latter part of the study, which impacted the larger channels, even though the remainder of the catchment and associated terrestrial vegetation began to recover.

Differences in response among severely burned stream sizes

There was a differential response between streams of different size. The two headwater streams (first and second order) responded sooner and recovered faster, in terms of the condition of both the habitat and the biota. The third- and fourth-order sites were still undergoing substantial change at the end of the 10 years. In fact, they changed more in 1996–1997, as a result of increased precipitation and runoff, than they did either in immediate response to the fire or in the next several years. The differential response between the smaller (first and second order) and larger (third and fourth order) streams to above-average precipitation probably was due to differences in water volume and sediment supply. The smaller streams, having smaller catchments and supplies of bed material (due to their closer proximity to bedrock), tended to downcut to bedrock and to braid; as a consequence, the zoobenthos remained relatively stable in the latter half of the study decade. In contrast, the larger streams tended to expand laterally, exposing additional amounts of loose alluvial deposits, which, combined with deep deposits of bedload, resulted in unstable channel conditions as waves of material moved through these stream reaches.

For all stream sizes, the response trajectory was more varied and less certain than previously thought (Minshall et al. 1989). However, this is to be expected for nonequilibrium, disturbance-driven ecosystems and is part of the challenge in seeking to understand their dynamics. Although conditions varied with stream size, our expectation that the effects of fire on total macroinvertebrate density or biomass would be attenuated progressively with distance downstream did not occur. This hypothesis was based on the assumption that downstream effects of wildfire would be decreased by the entrance of an increasing number of tributaries from unburned catchments that would gradually decrease the percentage of the overall catchment burned and dilute the fire effects with increasing stream size. In the present study, however, the percentage of catchment burned was relatively

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constant among the stream sizes examined, and thus, no attenuation effect occurred.

Differences in responses of macroinvertebrates with respect to burn extent

Although all of the Cache Creek sites were impacted by the 1988 fire, the benthic macroinvertebrates in the less extensively (<50%) burned catchments showed less of an effect than those in the more extensively (>60%) burned catchments. Thus, although we did not see a progressive decrease in impact on macroinvertebrates with respect to stream size, these results support our original assumption that the degree of disturbance to stream ecosystems by wildfire should be a function of the proportion of the catchment burned. In addition, the results suggest that, among streams of similar size, a negative relationship exists between the percent area burned and the degree of various biotic responses (e.g., macroinvertebrate assemblage composition, density, and biomass).

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Production of stream habitat gradients by montane watersheds: hypothesis tests based on spatially explicit path analyses

Daniel J. Isaak and Wayne A. Hubert

Abstract: We studied how the features of mountain watersheds interact to cause gradients in three stream attributes: baseflow stream widths, total alkalinity, and stream slope. A priori hypotheses were developed before being tested in a series of path analyses using data from 90 stream reaches on 24 second- to fourth-order streams across a fifth-order Rocky Mountain watershed. Because most of the conventional least squares regressions initially calculated for the path analyses had spatially correlated residuals (13 of 15 regressions), spatially explicit regressions were often used to derive more accurate parameter estimates and significance tests. Our final working hypotheses accounted for most of the variation in baseflow stream width (73%), total alkalinity (74%), and stream slope (78%) and provide systemic views of watershed function by depicting interactions that occur between geomorphology, land surface features, and stream attributes. Stream gradients originated mainly from the unidirectional changes in geomorphic features that occur over the lengths of streams. Land surface features were of secondary importance and, because they change less predictably relative to the stream, appear to modify the rate at which stream gradients change.

Résumé : Notre étude vise à comprendre comment les caractéristiques des réseaux hydrographiques de montagne interagissent pour former des gradients dans trois des descripteurs des cours d'eau : la largeur du lit au débit de base, l'alcalinité totale et la pente. Nous avons élaboré des hypothèses a priori avant de les éprouver dans des analyses de pistes causales basées sur des données provenant de 90 sections de 24 cours d'eau d'ordres 2 à 4, appartenant à un système hydrographique d'ordre 5 des Rocheuses. Parce que les régressions conventionnelles par la méthode des moindres carrés calculées initialement pour l'analyse des pistes possèdent des résidus qui présentent entre eux des corrélations spatiales (13 des 15 régressions), nous avons utilisé des régressions spatialement explicites pour obtenir des estimations plus précises des paramètres et de meilleurs tests de signification. Nos hypothèses de travail finales expliquent la plus grande partie de la variation dans la largeur du lit au débit de base (73%), l'alcalinité totale (74%) et la pente du cours d'eau (78%); elles génèrent une représentation du fonctionnement du système hydrographique qui décrit les interactions entre la géomorphologie, les faciès terrestres et les caractéristiques du cours d'eau. Les gradients dans les cours d'eau apparaissent surtout à cause de changements unidirectionnels des caractéristiques géomorphologiques le long du cours. Les faciès terrestres ont une importance secondaire et, parce qu'ils changent de façon moins prévisible en rapport avec le cours, ils semblent modifier le rythme auquel les gradients des cours d'eau évoluent.

[Traduit par la Rédaction]

Introduction

Stream ecologists have over the last century described patterns in lotic biotas relative to habitat gradients in streams (Shelford 1911; Huet 1959; Ward 1986). The ubiquity of these patterns led to the formulation of the River Continuum Concept (Vannote et al. 1980), which in part posits that lotic communities are structured by the quality and quantity of energy inputs to a stream and that these inputs result from interactions between the riparian zone and environmental gradients within a stream. The River Continuum Concept has become a dominant paradigm within stream ecology, but despite its explicit recognition of factors external to the stream and a trend toward broadscale management of stream systems, relatively few studies have examined linkages between stream habitats and surrounding landscapes.

Much of the work that links streams to their surroundings was conducted during the mid-twentieth century by geomorphologists working on stream energy expenditure theory (e.g., Leopold and Maddock 1953), and many authors have linked channel characteristics to broadscale variables such as watershed size or basin relief (Langbein 1947; Strahler 1957). More recently, fisheries scientists have related fish habitats to geomorphic and geologic traits of watersheds (Lanka et al. 1987; Nelson et al. 1992), while hydrologists and geohydrochemists have made similar advances in explaining stream flows (Thomas and Benson 1970; Zecharias and Brutsaert 1988) and water chemistries (Teti 1984; Close and Davies-Colley 1990). But despite the merit of these efforts, previous studies have oversimplified stream–watershed

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linkages by not including all of the factors that may affect a particular stream attribute in the same model. Additionally, no study has modeled the chains of causality that can be expected to link watershed features to instream habitats. We attempted to partially fill these voids by examining how watershed features interact to impinge upon streams and cause gradients in three habitat attributes important to lotic biotas: baseflow stream width, total alkalinity, and stream slope. Our objectives were to develop detailed hypotheses about the linkages between watersheds and stream attributes, use empirical data to test predictions generated by the hypotheses, and revise the hypotheses to derive an accurate and systemic understanding of how watersheds function to produce stream habitat gradients.

Stream gradient hypotheses

In this section, the hypothesis for each stream attribute is described and rationales are provided for the causal relationships among variables. To facilitate accurate modeling of these systems, the features characterizing the terrestrial landscape were divided into two classes. Geomorphic features pertain to size, shape, or orientation, whereas land surface features describe surficial or near-surface aspects of a watershed. Hypothesis 1 depicts the predicted causal structure among factors associated with baseflow stream width (Fig. 1), a variable that provides a measure of habitat volume during periods when competitive interactions are expected to be strongest (e.g., Gipson 1973). Stream width also helps determine the trophic status and community structure of streams by regulating the amount of sunlight incident upon a stream (Vannote et al. 1980).

A survey of the literature, combined with observations made while working in the field, suggested that baseflow widths were potentially affected by nine factors: cattle density, watershed size, aquifer storage, basin elevation, drainage density, watershed slope, tree abundance, soil compaction, and road density. In a mechanistic sense, baseflow widths should be positively affected by watershed size because larger land areas collect and concentrate more precipitation. Elevation is also expected to be an important determinant of stream flows in montane landscapes because the lapse rate of temperature with elevation and orographic lifting of air parcels cause greater condensation and precipitation at high elevations (Brooks et al. 1991). Therefore, if all other factors are held constant, the baseflow width of a stream flowing from a high-elevation basin should be greater than the width of a stream flowing from a low-elevation basin.

Once a water molecule enters a watershed, its probability of contributing to baseflow is affected by factors that alter the timing and intensity of precipitation runoff. Watersheds underlain by large amounts of geologic materials that provide aquifer storage should have greater baseflows because more precipitation will enter long-term storage and not immediately pass through the system. Conversely, land uses such as livestock grazing or timber harvest can expedite runoff and negatively affect baseflows by compacting soil layers and decreasing infiltration (Brooks et al. 1991; Meehan 1991). Watershed slope, road density, and drainage density are also expected to negatively affect baseflows because denser stream and road networks and greater hydraulic gradients in steep watersheds increase the throughput of precipitation (Carlston 1963; Zecharias and Brutsaert 1988; Jones and Grant 1996). Drainage density is in turn predicted to be positively affected by basin elevation and watershed slope because more precipitation will be available to form stream channels at high elevations, and this precipitation will be quickly concentrated in watersheds with steep slopes, thereby enhancing channel initiation and maintenance (Langbein 1947).

Evapotranspiration allows water to exit basins by a route other than stream flow (Brooks et al. 1991). Because trees transpire at greater rates than other vegetation types (Brown and Thompson 1965), watersheds with more trees should have lower baseflows. Tree abundance is in turn expected to be controlled by geomorphic characteristics that produce the mesic conditions needed by trees (Running 1984), and we predict that tree abundance will be positively related to basin elevation. Watershed slope is also expected to have a positive effect on tree abundance because topographic shading is greater in steeper watersheds.

The factors described above affect stream width by altering the amount of water in the channel. However, grazing by cattle and the associated bank destabilization can affect the physical structure of the channel (Belsky et al. 1999), and it is predicted that streams will be wider where cattle densities are greater. Although the distribution of cattle across a landscape is somewhat arbitrary given human intervention, our field observations suggest that cattle are generally grazed in areas amenable to their preferences for gradual slopes (Mueggler 1965) and grass forage (Odion et al. 1988). Grass abundance is expected to be controlled by the same geomorphic factors that regulate the abundance of trees, although slope and elevation should have negative rather than positive effects on grass abundance.

In the second hypothesis, we describe linkages among watershed features and stream alkalinity (Fig. 1), a measure of the nutrient content of water that has often been positively correlated with greater abundances of aquatic macroinvertebrates and fish (e.g., Krueger and Waters 1983; Kwak and Waters 1997). Although a causal relationship between alkalinity and stream biota is not well substantiated, speculations are that organisms in more alkaline waters use less energy for ionic regulation (Fiance 1978) or that alkalinity ions provide an important source of dissolved inorganic carbon that stimulates greater primary productivity (Krueger and Waters 1983).

In the pH range of most surface waters, alkalinity is determined almost entirely by the abundance of bicarbonate ions, which originate from the chemical weathering of bedrock-derived mineral soils by carbonic acid dissolved in groundwater (Drever 1997). Stream alkalinity should therefore be positively related to the abundance of carbonate bedrocks (i.e., limestone and dolomite) because these geologic materials weather into soils that can produce large amounts of bicarbonate ions (Drever 1997). Soil development is expected to relate to alkalinity because thick and finely dissected soils provide more surface area to yield bicarbonate ions, and these soils will lengthen soil–water contact time by slowing the movement of water through a hillslope (Drever and Zobrist 1992). Consequently, steep watersheds should have streams with low alkalinities because soil depth is inversely

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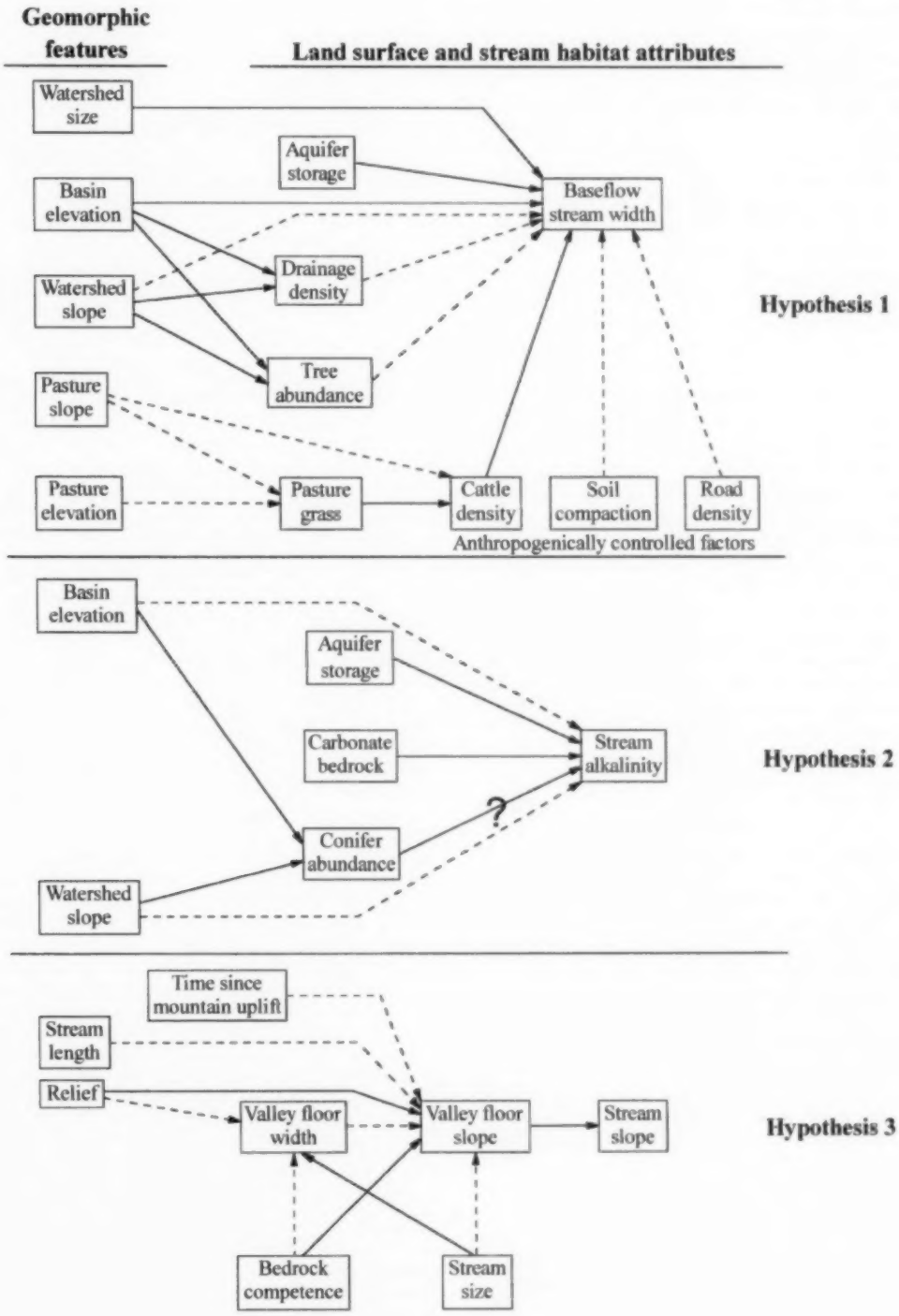
Fig. 1. Hypotheses about factors that cause habitat gradients in small mountain streams. Arrows point in the direction of causality. If an arrow has a solid line, the relationship is predicted to be positive; if an arrow has a broken line, the relationship is predicted to be negative.



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related to slope steepness (Carson and Kirkby 1972). Basin elevation is also predicted to negatively affect alkalinity because colder temperatures at high elevations slow biological and chemical processes that build soils (Drever and Zobrist 1992). Conversely, the amount of aquifer storage within a watershed should have a positive effect on alkalinities because greater storage will lengthen soil–water contact time and provide more opportunity for bicarbonate ions to enter solution.

The abundance of coniferous trees may affect alkalinity because the decomposition of conifer needles results in the development of acid soils (Nihlgard 1970). Water percolating through these soils becomes more acid, but the effect that this has on stream alkalinity depends on the composition of the underlying bedrock. In watersheds with carbonate substrates, low pHs increase weathering rates, which releases additional bicarbonate ions and leads to the alkalization of surface waters (Kilham 1982). But in watersheds overlying less soluble igneous or metamorphic bedrocks, weathering rates will not change appreciably, and stream alkalinities may actually decrease as greater acidities use some of the buffering capacity already present in the water (e.g., Sharpe et al. 1984).

Hypothesis 3 depicts the predicted causal structure between watershed features and reach-scale stream slope (Fig. 1), a variable that strongly affects the structure of habitats (Montgomery and Buffington 1997) and the attributes of lotic biotas (Isaak and Hubert 2000). The stream slope hypothesis differs from the previous two hypotheses in that the primary response variable is predicted to be entirely controlled by one factor, the slope of the underlying valley floor. As such, understanding how the longitudinal profiles of valley floors are formed should provide insight into the factors determining reach-scale stream slope.

During mountain-building episodes, uplift rates exceed the erosive capacity of a stream, but the magnitude of this exceedance decreases in a downstream direction as stream size and erosive capacity increase (Snow and Slingerland 1990). Downstream areas, therefore, are more quickly reduced than upstream areas, which results in the concave upward profile of most valley floors (Wheeler 1979) and suggests that stream size will negatively affect valley floor slope. Stream length is expected to have a similar negative effect on valley floor slope because the descent to baselevel can be spread over a greater distance in a longer stream. The amount of time since the mountain-building event is also predicted to have a negative effect on valley floor slope because weathering and erosive forces will gradually reduce the relief of a mountain range. Conversely, valley floor slope should be steeper where relief is greater because the amount of geologic material to be eroded increases as uplift magnitude increases.

The rate at which geologic materials weather and become susceptible to fluvial displacement can affect valley floor slope at two spatial scales. At the scale of the entire slope profile, greater bedrock competence will slow the rate at which a landmass erodes and should increase the steepness of the profile (Wheeler 1979). At a more restricted scale, variation in the competence of the individual geologic formations that a stream flows across can alter the width of a valley floor (e.g., Hupp 1982). Valley floor width will in turn have a negative effect on valley floor slope because streams can deposit more alluvium and build gently sloping floodplains in wider valleys. Valley floor width should be positively affected by stream size because larger streams not only carve wider valleys but transport and deposit more alluvium. Finally, relief is expected to have a negative effect on valley floor width because streams farther removed from baselevel are more likely to be downcutting and will create steep and incised valleys rather than alluvial valleys.

Materials and methods

Study area

Data to test the hypotheses were collected from the 2150-km^2 Salt River watershed on the border between Idaho and Wyoming (42°37′30″ latitude, 110°52′30″ longitude). The Salt River watershed is part of the Middle Rocky Mountain physiographic province (Fenneman 1931) and is bordered by mountain ranges that differ markedly in morphology (Fig. 2). On the east side of the watershed, the rugged Salt River Range peaks at elevations exceeding 3300 m. The terrain in the Caribou and Webster ranges to the west and the Gannett Hills to the south is less rugged and elevations do not exceed 2800 m. Mountain massifs consist of uplifted sedimentary rock strata that have been convoluted to yield a complex bedrock geology comprising more than 40 geologic formations (Mitchell and Bennett 1979; Oriel and Platt 1980). Each formation contains a mixture of interbedded rock types that consists of various mudstones, conglomerates, shales, sandstones, limestones, and dolomites. Karstic structures occur within some of the formations, but obvious outflows from these structures affected none of the streams included in this study. Formation dates of the geologic formations range widely from the Mississippian to the Cretaceous ages, but the complement of rock types within the four mountain ranges exhibit distinct age distributions. Surficial geology consists primarily of colluvium overlain by thin soils, but bedrock outcrops are not uncommon hillslope features, and extensive alluvial deposits of Quaternary age fill the main river valley and downstream portions of some mountain valleys. Pleistocene glaciation occurred at the highest elevations in the Salt River Range, but did not extend to valley bottoms (Oriel and Platt 1980).

Characteristics of mountain streams differ with contrasts in mountain morphologies. Valleys in the Salt River Range are narrow and constrained, whereas valleys throughout other mountain ranges grade from constrained in upstream areas to unconstrained in downstream areas. Salt River Range streams have straighter channel patterns, larger substrate sizes, and steeper slopes than other streams. Hydrographs of all streams are typical for the Rocky Mountain region, with peak discharges driven by snowmelt in late May and June, followed by baseflows from late July into March.

The climate in the Salt River watershed is classified as cold with humid winters. Mean annual air temperature on the Salt River valley floor is 3.5°C, and monthly averages range from –9.1°C in January to 16.7°C in July (Owenby and Ezell 1992). Precipitation is evenly distributed throughout the year and comes as snow during cooler months and as rain during the remainder of the year. Annual precipitation averages 50 cm on the Salt River valley floor and over 100 cm at high elevations (Othberg 1984).

Land use in the Salt River watershed is indicative of geomorphic constraints and land ownership. Native grass communities in the privately owned main valley and the lower ends of alluviated mountain valleys have been converted to personal residences, pastures, and grain fields. Mountainous areas are owned and managed by the U.S. Forest Service and are used primarily for livestock grazing and recreation. Sheep grazing is common at the highest elevations, whereas cattle grazing is confined to the Gannett Hills and lower elevations in the Caribou and Webster ranges. Forestry

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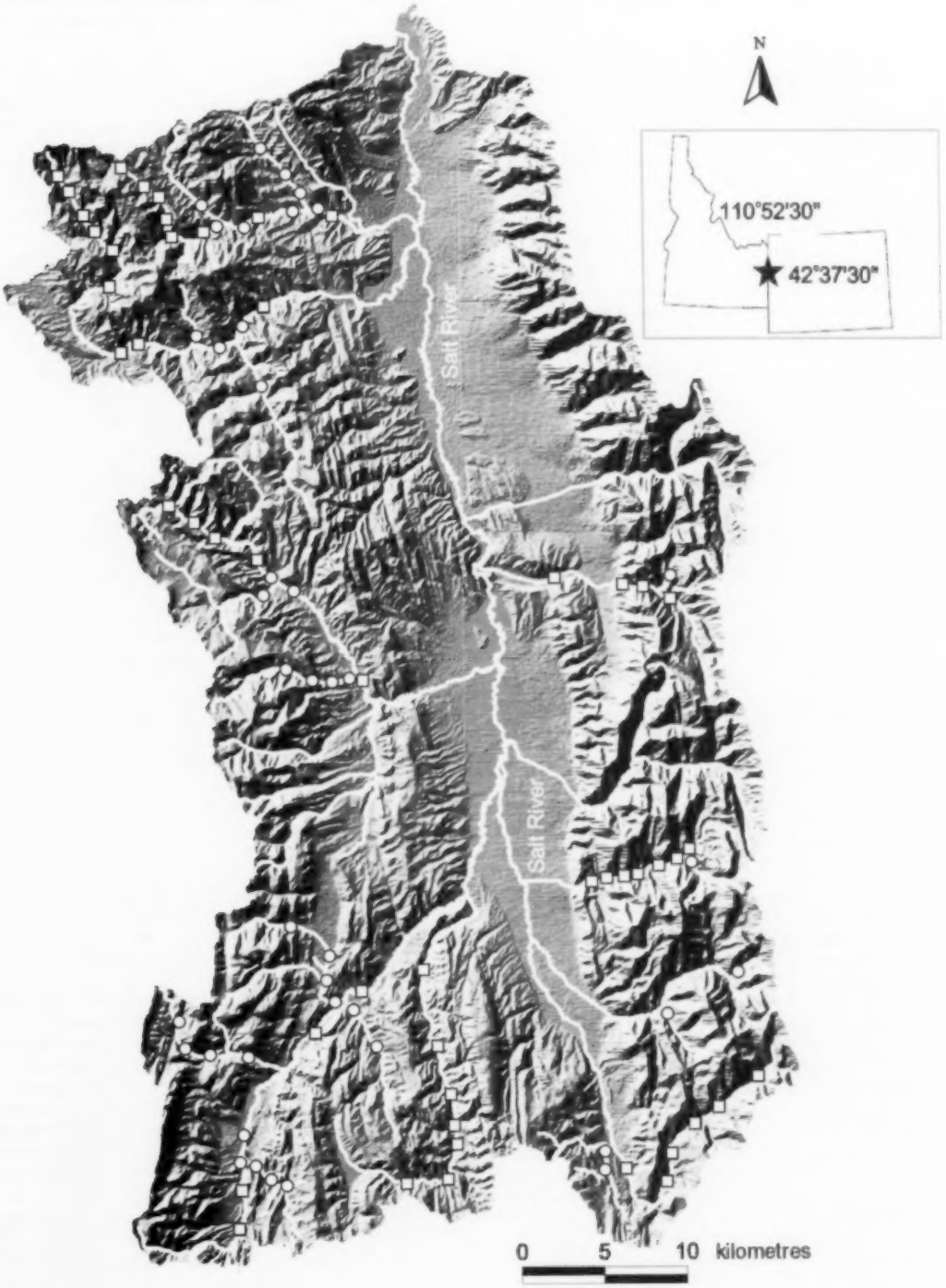
Fig. 2. Greyscale hillshade of a digital elevation model depicting the sample reaches and stream drainage network across the Salt River watershed. Locations marked with squares were sampled in 1996, and circles denote reaches sampled in 1997. The inset map shows the study area location on the border between Idaho and Wyoming, U.S.A.



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activities have never constituted a major land use within the watershed, but unpaved roads have been constructed in most large drainages.

Data collection

Sampling was conducted at 50 reaches in 1996 and 40 reaches in 1997 on 24 second- to fourth-order streams (Fig. 2). Each reach consisted of a section of stream with consistent slope that averaged 180 m in length (range = 63–465 m). Sampling began in early July when snowmelt runoff subsided and continued until the middle of September when air temperatures cooled. A reach was sampled at every 50-m change in elevation along the length of a stream, and two reaches were sampled on the same stream near major tributary confluences: one upstream from the confluence and one downstream. Stream surveys began at the downstream end of each stream and progressed upstream except when time was limiting, in which case, one reach was sampled at the lower end of a stream (eight of 24 streams). No reaches were sampled downstream from reservoirs, major water withdrawals, or significant spring inputs, as these features would artificially alter gradients in stream habitats.

At each stream reach, wetted stream width was measured to the nearest centimetre along transects spaced at 10-m intervals and run perpendicular to the direction of stream flow, total alkalinity was measured with a water chemistry kit (test kit 24443-00, Hach, Loveland, Co.; mention of trade names does not imply endorsement by the University of Wyoming), and stream slope was measured with an Abney level as described in Isaak and Hubert (1999). Reach locations were identified using available landmarks and recorded on 1 : 24 000 scale topographic maps for later digitization.

Land surface and geomorphic variables were quantified for the watersheds upstream from sample reaches using a geographic information system (GIS). Coverages included 30-m resolution slope and elevation models, 1 : 24 000 scale stream hydrology, road, and vegetation coverages, a point coverage of reach locations, a 1 : 250 000 scale bedrock geology coverage, and a polygon coverage of U.S. Forest Service grazing allotment boundaries that contained information on the number of cattle and sheep grazed in each allotment. Completed coverages or data required to build them were acquired from the Caribou and Bridger–Teton national forests, published geologic maps (Mitchell and Bennett 1979; Oriel and Platt 1980), the Spatial Data and Visualization Center (2000) at the University of Wyoming, the Idaho Department of Water Resources (2000), and the Geospatial Data Center (2000), and the Idaho GAP Analysis Project (2000) at the University of Idaho.

The calculation of most land surface and geomorphic variables is described in detail in Isaak and Hubert (2001) and brief descriptions are provided in Table 1. Some variable constructs, however, require additional explanation. Grazing by sheep and cattle, for example, was the only land use at the study area with the potential to compact soil layers across broad areas. As such, soil compaction was estimated by dividing the total weight of domestic livestock (mean weights of 55 kg for sheep and 320 kg for cows were used) that grazed each watershed by the size of the watershed. The aquifer storage variable was impossible to quantify directly because of an absence of data on the water-holding capacities of geologic formations. Instead, we used the area of unconsolidated Quaternary alluvial deposits divided by watershed area as a surrogate measure because these deposits provide significant inputs of water to streams during baseflow periods (Smuin 1990) and are conspicuously depicted on geologic maps. Cattle density, pasture grass, pasture slope, and pasture elevation were quantified at the scale of a grazing allotment in accord with the resolution at which these data were available. Additionally, pasture grass was calculated by dividing the allotment area covered by grassland at an elevation lower than 2400 m by the area of the grazing allotment. The elevation limit was imposed because alpine meadows often exist above the treeline, but sheep were grazed in these areas rather than cattle.

In the stream slope hypothesis, valley floor slope was quantified from 1 : 24 000 scale topographic maps by digitizing the distance of the valley floor between the elevation contours that bounded a stream reach and dividing this distance by the concurrent drop in elevation. Using Grant and Swanson's (1995) definition of a valley floor as the valley width at an elevation 3 m above the streambed, valley floor width was calculated as an average from three elevation transects that were evenly spaced over the length of a reach and were interpolated from the elevation model. The complexities of the bedrock geology underlying the study area and an absence of weathering rate data made it impossible to quantify the effect that the competence of the bedrock adjacent to each sample reach would have on valley floor width. However, we were able to estimate the effect of bedrock competence on valley floor slope using a surrogate measure based on the rank-ordered age of the set of geologic formations that composed each mountain range. This measure was predicated on the belief that the oldest sedimentary rocks would have been overlain by the greatest amount of overburden and become most strongly lithified.

Data analysis

Path analysis (Wright 1934; Mitchell 1993) was used to analyze each hypothesis. To conduct each path analysis, regressions were calculated for response variables in a manner that was structured by the hypothesis. Five regressions were required for the initial path analysis of Hypothesis 1, one each for baseflow stream width, cattle density, grass abundance, tree abundance, and drainage density (Fig. 1). The direct effect (DE), or the amount of change in a response variable attributable to a predictor, was estimated as a partial regression coefficient that was standardized to facilitate comparisons of effect strengths within and among the different regressions that composed a hypothesis (unstandardized coefficients can be found in Isaak (2001)). The overall accuracy of a hypothesis was assessed by comparing the correlation matrix predicted by a hypothesis with the correlation matrix derived from the empirical data (Table 2). Predicted correlations were calculated as described in Sokal and Rohlf (1995) and plotted against observed correlations before the strength of this bivariate relationship was quantified with a simple Pearson correlation. We also used procedure CALIS (SAS Institute Inc. 1989) to calculate a χ^2 measure of model fit for each hypothesis. The χ^2 statistic was slightly biased because the CALIS procedure used aspatial parameter estimates rather than spatially explicit parameter estimates (discussed below), but it still provided a useful tool for assessing the accuracy of each hypothesis.

Assumptions regarding the distribution of residual errors derived from the regression models were checked using tests for normality and homoscedasticity, and departures from linearity were assessed using scatterplots and residual plots. If assumptions were violated, the necessary variable transformations were applied (see Table 2). Linear regression models also require that residuals be independent, but temporally correlated residuals could occur where response variables exhibited trends in time over the course of data collection efforts (e.g., baseflow stream widths decreasing during a sample season due to gradual declines in aquifer discharge). To test for time effects, sample year (interyear variation), sample date (intrayear variation), and both year and date terms were iteratively included as predictors in the appropriate regressions. Where time effects were apparent, these terms were retained to control for this nuisance variation.

Given the spatial dimension of the data set, violations of the independence assumption were also possible if the size of regression residuals were related to the distance between sampling locations. The residuals derived from least squares regressions were tested for spatial correlation using Moran's I (Moran 1948) and a row standardized spatial weights matrix based on the inverse of the stream distance between sample locations. Results using a spatial weights matrix based on the inverse of straight-line distance were

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Table 1. Descriptive statistics for variables in a data set used to test hypotheses about factors that cause habitat gradients in small mountain streams.

Variable[a]	*N*	Mean	SD	Minimum	Maximum
CATTLE	90	6.2	10.5	0.0	37.5
PAS_SLOP	90	15.9	4.9	1.0	26.0
PAS_GRAS	90	28.4%	24.3%	1.6%	100.0%
PAS_ELE	90	2246	204	1892	2649
WAT_SIZE	90	40.9	41.4	1.6	256.1
BAS_ELE	90	2394	178	2124	2904
WAT_SLOP	90	17.0	4.2	10.6	27.1
D_DEN	90	1.33	0.18	0.80	1.70
ROADS	90	0.50	0.29	0.0	1.07
AQUIFER	90	4.11%	5.31%	0.0%	23.8%
SOIL_COM	90	3056	1831	519	8352
TREE	90	67.0%	13.4%	30.6%	91.2%
STR_WID	90	4.33	1.66	1.25	8.84
CONIFER	90	42.9%	14.4%	16.3%	78.0%
CARBNAT	90	53.1%	26.1%	0.0%	100.0%
STR_ALK	90	197	42	82	286
BEDROCK	90	2.33	1.12	1	4
VFLR_WID	90	96.8	82.6	40	603.3
R_RELIEF	90	0.36	0.15	0.09	0.81
STR_LENG	90	82.0	32.3	24.0	126.7
VFLR_SLOP	90	2.82%	1.83%	0.48%	9.60%
STR_SLOP	90	2.20%	1.67%	0.19%	10.14%

[a]CATTLE, cattle density within a grazing allotment (cows·km^{-2}); PAS_SLOP, mean grazing allotment slope (°); PAS_GRAS, proportion of a grazing allotment covered by grassland at an elevation of less than 2400 m; PAS_ELE, mean grazing allotment elevation (m); WAT_SIZE, watershed size (km^2); BAS_ELE, mean basin elevation (m); WAT_SLOP, mean watershed slope (°); D_DEN, density of streams within a watershed (km·km^{-2}); ROADS, density of roads within a watershed (km·km^{-2}); AQUIFER, proportion of a watershed with Quaternary alluvial deposits; SOIL_COM, total weight of domestic livestock grazed within a watershed divided by watershed size (kg·km^{-2}); TREE, proportion of a watershed covered by trees; STR_WID, wetted stream width at reach location (m); CONIFER, proportion of a watershed covered by conifers; CARBNAT, proportion of a watershed with carbonate bedrock; STR_ALK, total stream alkalinity at reach location (mg·L^{-1}); BEDROCK, bedrock competence (rank ordered age of geologic materials composing four mountain ranges); VFLR_WID, width of valley floor adjacent to a reach (m); R_RELIEF, elevation difference between a reach and the mouth of the Salt River (km); STR_LENG, stream distance between a reach and the mouth of the Salt River (km); VFLR_SLOP, slope of valley floor underlying a reach; STR_SLOP, water surface slope of a reach.

nearly identical and are not reported. When spatial non-independence was detected, SpaceStat 1.90 (Anselin 1998) was used to estimate spatially explicit maximum likelihood regressions that took the spatial juxtaposition of observations into account and thereby minimized or eliminated the lack of independence among residuals. Because the spatial regressions minimized violations of the independence assumption, they should have provided the most accurate results, and inference was based on spatial regressions when these models were estimated. To facilitate model comparisons, Akaike's Information Criterion (AIC) (Akaike 1981) was calculated and R^2 values were calculated as the squared correlations between predicted and observed values.

Multicollinearity, which results from correlations among the predictor variables in a multiple regression, can lead to unreliable parameter estimates and significance tests when correlations are strong. The effects of multicollinearity were monitored using standard diagnostic tests such as variance inflation factors, condition indices, and tolerance values (SAS Institute Inc. 1989). When problems arose, the predictor variable or variables most responsible were either deleted or included with subsets of predictors in an alternative regression for which multicollinearity problems were less severe.

Results

Much of the variation in baseflow stream widths was explained by predictors included in the initial hypothesis (R^2 = 0.74) (Fig. 3, 1A). Stream widths were affected most by a large positive effect from watershed size (DE = 0.63, $p < 0.01$), a negative effect from soil compaction (DE = –0.29, $p < 0.01$), and small positive effects from aquifer storage (DE = 0.13, $p = 0.05$), basin elevation (DE = 0.15, $p = 0.04$), and tree abundance (DE = 0.18, $p = 0.01$). The effects of road density and watershed slope on baseflows were not estimated due to multicollinearity problems that resulted from strong correlations between watershed slope and several other predictors and between roads and basin elevation. For other response variables in this hypothesis, the abundance of grass within a pasture had much of its variation explained by moderate negative effects from pasture slope (DE = –0.39, $p < 0.01$) and pasture elevation (DE = –0.49, $p < 0.01$). In turn, grass abundance had a positive effect on cattle density (DE = 0.23, $p = 0.07$). Problems with multicollinearity in the regression for tree abundance led to the deletion of basin elevation as a predictor variable, but watershed slope had a positive effect on trees (DE = 0.25, $p = 0.04$). Finally, drainage density was unaffected by either watershed slope (DE = –0.01, $p = 0.99$) or basin elevation (DE = –0.15, $p = 0.45$).

As would be expected given the number of small and insignificant effects in the original stream width hypothesis,

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Table 2. Correlations among variables in a data set used to test hypotheses about factors that cause habitat gradients in small mountain streams.

Variable	CATTLE	PAS_SLOP	PAS_GRAS[a]	PAS_ELE	WAT_SIZE	BAS_ELE	WAT_SLOP	D_DEN[a]	ROADS	AQUIFER	SOIL_COM
CATTLE	—										
PAS_SLOP	**–0.49**	—									
PAS_GRAS[a]	**0.52**	**–0.76**	—								
PAS_ELE	**–0.42**	**0.77**	**–0.67**	—							
WAT_SIZE	0.11	**–0.32**	**0.28**	**–0.39**	—						
BAS_ELE	**–0.33**	**0.63**	**–0.48**	**0.82**	**–0.34**	—					
WAT_SLOP	**–0.39**	**0.72**	**–0.61**	**0.60**	**–0.21**	**0.77**	—				
D_DEN[a]	0.05	**–0.30**	0.13	**–0.24**	**0.22**	**–0.23**	**–0.26**	—			
ROADS	**0.42**	**–0.58**	**0.51**	**–0.63**	0.11	**–0.72**	**–0.68**	0.03	—		
AQUIFER	**0.29**	**–0.37**	**0.50**	–0.20	**0.37**	–0.10	**–0.22**	0.18	0.00	—	
SOIL_COM	**0.46**	**–0.53**	**0.67**	**–0.33**	0.08	**–0.39**	**–0.69**	0.11	**0.52**	**0.23**	—
TREE	**–0.20**	0.18	**–0.31**	**–0.21**	–0.05	**–0.26**	0.11	0.03	**0.21**	**–0.39**	**–0.26**
STR_WID	–0.12	0.02	–0.17	–0.07	**0.67**	0.01	**0.22**	0.20	**–0.26**	**0.23**	**–0.39**
CONIFER[a]	**–0.32**	**0.64**	**–0.27**	**0.41**	**–0.24**	**0.55**	**0.69**	**–0.43**	**–0.33**	–0.11	**–0.34**
CARBNAT	0.01	0.16	–0.08	0.09	0.01	**0.22**	**0.44**	0.03	–0.04	0.11	–0.19
STR_ALK	**0.44**	**–0.54**	**0.56**	**–0.55**	**0.23**	**–0.70**	**–0.68**	**0.23**	**0.66**	**0.23**	**0.53**
BEDROCK	–0.15	**0.51**	–0.17	**0.48**	–0.12	**0.62**	**0.69**	**–0.41**	**–0.40**	0.15	**–0.30**
VFLR_WID[a]	0.09	**–0.35**	0.16	**–0.33**	**0.45**	–0.15	–0.07	0.18	0.06	**0.26**	–0.08
R_RELIEF	**–0.25**	**0.38**	**–0.28**	**0.73**	**–0.49**	**0.80**	**0.32**	–0.17	**–0.43**	–0.11	–0.04
STR_LENG	0.15	0.07	**0.26**	**0.40**	–0.10	**0.48**	0.09	**–0.21**	–0.08	**0.30**	**0.33**
VFLR_SLOP[a]	**–0.28**	**0.45**	**–0.37**	**0.45**	**–0.69**	**0.46**	**0.38**	**–0.33**	**–0.32**	**–0.45**	–0.20
STR_SLOP[a]	**–0.25**	**0.48**	**–0.42**	**0.46**	**–0.69**	**0.45**	**0.43**	**–0.40**	**–0.27**	**–0.41**	**–0.24**

Note: Correlation values in bold type have probability values less than 0.05 and were calculated assuming no spatial correlation. See Table 1 for definitions of variables.

[a]Transformation applied to meet regression assumptions. PAS_GRAS = $\sqrt{x}$, D_DEN = $(x^{2.63} - 1)/2.63$, CONIFER = arcsin x, VFLR_WID = $-1/x$, VFLR_SLOP = $\log_{10} x$, and STR_SLOP = $(x^{0.115} - 1)/0.115$.

the data structure that it predicted differed strongly from the structure observed in the empirical data (χ^2 = 170.35, df = 35, $p < 0.01$). A scatterplot of the observed and predicted correlations also indicated that, despite a strong bivariate correlation (r = 0.93, $p < 0.01$), many correlation pairs plotted well away from the 1:1 line (Fig. 3, 1A).

The stream width hypothesis was revised by deleting drainage density and cattle density and using basin elevation rather than watershed slope as a predictor of tree abundance (Fig. 3, 1B). Additionally, road density was included as a predictor of stream width, and the linkage to basin elevation was dropped to avoid problems with multicollinearity. After these revisions, Hypothesis 1B was simpler than its predecessor and it explained a similar amount of variation in baseflow widths (R^2 = 0.73). A large decrease in the difference between the predicted and observed correlation matrices (χ^2 = 40.54, df = 6, $p < 0.01$) suggested that 1B was an improvement over 1A, but both of the new paths included in this hypothesis were of negligible importance.

In a further revision of the stream width hypothesis, road density and the path from basin elevation to tree abundance were deleted and paths were reinserted from watershed slope to tree abundance and from basin elevation to stream width (Fig. 3, 1C). The overall fit of 1C was similar to 1B (χ^2 = 53.38, df = 6, $p < 0.01$), but it seemed a more plausible representation of the study system because all of the predictors had statistically improbable effects.

In the alkalinity hypothesis, multicollinearity problems masked the effect of basin elevation when it was included with watershed slope in the initial regression. Therefore, the pathway from basin elevation to alkalinity was dropped and this regression was recalculated. The four remaining predictors accounted for 72% of the variation in stream alkalinity (Fig. 4, 2A), mostly due to a negative effect from watershed slope (DE = –0.44, $p < 0.01$) and a small positive effect from carbonate bedrock (DE = 0.19, $p < 0.01$). Stream alkalinity appeared to be unaffected by aquifer storage (DE = –0.03, p = 0.67) or conifer abundance (DE = –0.07, p = 0.43). The bivariate relationship between the correlations predicted by this hypothesis and the empirically derived correlations was strong (r = 0.96, $p < 0.01$), although the χ^2 statistic suggested some discrepancies (χ^2 = 12.31, df = 2, $p < 0.01$).

The alkalinity hypothesis was revised by dropping conifer abundance, aquifer storage, and the path between watershed slope and stream alkalinity. A path was added from basin elevation to alkalinity (Fig. 4, 2B), which, in the absence of watershed slope, proved to be a strong linkage (DE = –0.77, $p < 0.01$). In Hypothesis 2C, we reinserted the path between watershed slope and alkalinity because despite problems with multicollinearity, a model containing both causal factors seemed most realistic on theoretical grounds. In the absence of aquifer storage and conifer abundance, multicollinearity simply suppressed the estimated effect sizes of basin elevation (DE = –0.25, $p < 0.01$) and watershed slope (DE = –0.29, $p < 0.01$). Hypothesis 2C accounted for more of the variation in stream alkalinity than either of its predecessors (R^2 = 0.74), had effects that concurred with a priori predictions, and had closely matched data structures (r = 0.99, $p < 0.01$). A χ^2 test of overall model fit could not be calculated because no degrees of freedom are available for this test in a fully specified path model (i.e., paths exist from every predictor to every response variable).

In the slope hypothesis, valley floor slope had a large ef-

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TREE	STR_WID	CONIFER[a]	CARBNAT	STR_ALK	BEDROCK	VFLR_WID[a]	R_RELIEF	STR_LENG	VFLR_SLOP[a]	STR_SLOP[a]
—										
0.14	—									
0.25	–0.02	—								
0.24	0.10	**0.54**	—							
0.11	–0.18	**–0.38**	–0.02	—						
–0.14	0.08	**0.81**	**0.61**	**–0.36**	—					
0.03	**0.37**	**–0.24**	0.06	0.12	–0.08	—				
–0.42	**–0.33**	**0.23**	–0.05	**–0.52**	**0.32**	**–0.30**	—			
–0.55	**–0.26**	**0.32**	**0.27**	0.02	**0.57**	–0.18	**0.58**	—		
0.06	**–0.45**	**0.38**	–0.01	**–0.46**	**0.22**	**–0.49**	**0.49**	0.03	—	
0.09	**–0.45**	**0.39**	0.01	**–0.50**	**0.24**	**–0.40**	**0.45**	–0.01	**0.88**	—

fect on stream slope (DE = 0.88, $p < 0.01$) that explained most of its variation ($r^2 = 0.78$) (Fig. 5). In turn, much of the variation in valley floor slope was explained by large effects from stream length (DE = –0.69, $p < 0.01$) and relief (DE = 0.53, $p < 0.01$) and smaller effects from bedrock competence (DE = 0.45, $p < 0.01$), stream size (DE = –0.39, $p < 0.01$), and valley floor width (DE = –0.27, $p < 0.01$). Seventeen percent of the variation in valley floor width was explained by a positive effect from stream size (DE = 0.32, $p < 0.01$) and a smaller effect from relief (DE = –0.17, $p = 0.16$). In a modification of the a priori hypothesis, time since uplift was excluded from consideration because the mountain ranges at the study site were uplifted simultaneously. The χ^2 statistic suggested some difference between data structures ($\chi^2 = 15.02$, df = 7, $p = 0.04$), but a scatterplot of the predicted and observed correlations indicated only slight discrepancies ($r = 0.99$, $p < 0.01$). In a minor revision of this hypothesis, the weak path between relief and valley floor width was deleted, but no improvements in overall model fit were observed ($\chi^2 = 18.62$, df = 8, $p = 0.02$; $r = 0.98$, $p < 0.01$).

Spatially correlated residuals were common derivatives of conventional least squares regressions as indicated by the significance of Moran's *I* values for most of these regressions and the subsequent significance of autocorrelation coefficients in the spatial regressions (Table 3). Autocorrelation coefficients indicated that the strength of autocorrelation present in the regression data sets ranged widely, from nonexistent for stream slope and valley floor slope regressions, to moderately strong for four regressions of stream width and valley floor width, to very strong in the remaining nine regressions. In all cases, autocorrelation parameters were positive, indicating that proximal stream reaches contained similar information. The spatial regressions tended to decrease the magnitude of parameter estimates (8 increased, 27 decreased, and 3 remained constant), and the amount of change in parameter estimates was positively related to the strength of autocorrelation ($r = 0.66$, $p < 0.01$). AIC values always suggested that the spatial regressions were an improvement over the least squares regressions, and differences between the parameter estimates were sometimes large enough to qualitatively change inferences.

Discussion

The final versions of the hypotheses provide systemic views of watershed function by depicting interactions that occur between geomorphology, land surface features, and stream attributes. The hypotheses also suggest that most of the variation in baseflow stream widths, stream alkalinity, and stream slope can be explained by characteristics of the surrounding watershed. This result indicates a tight linkage between mountain streams and the terrestrial setting and allows insight into the origin of gradients in small mountain streams. Of the two classes of watershed descriptors, geomorphic features had the strongest effects on stream attributes, and gradients appear to arise primarily from the unidirectional changes in geomorphic features that occur over the lengths of streams. Land surface features were of secondary importance and, because they change less predictably relative to the stream, appear to modify the rate at which stream gradients change.

This study also provides inference regarding the relative importance of the specific causal factors affecting each stream attribute. In the stream width hypothesis, watershed size had a positive effect that was greater in magnitude than all other factors. Tree abundance, basin elevation, and aquifer storage had smaller positive effects. A positive effect from trees was unexpected given the frequency with which

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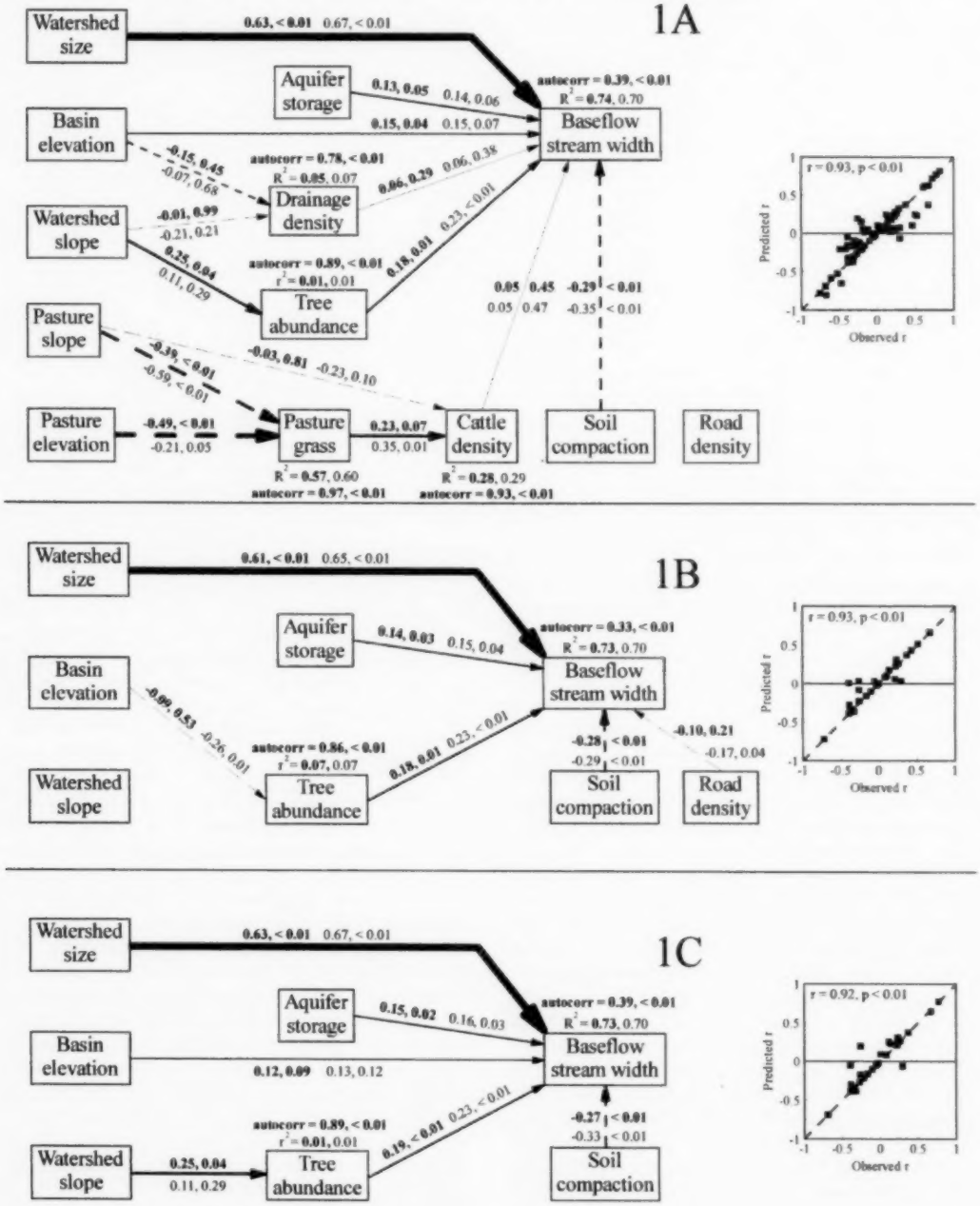
Fig. 3. Path diagrams for three versions of a hypothesis about factors that affect baseflow stream widths of small mountain streams. The width of an arrow is proportional to the strength of an effect that a predictor variable has on a response variable. Numbers next to arrows are standardized partial regression coefficients and probability values derived from spatially explicit regressions (bold type) and conventional least squares regressions. Numbers next to response variables are the autocorrelation coefficients for the spatial regressions and the variation explained by the spatial regressions (bold type) and least squares regressions.



negative effects have been observed (Bosch and Hewlett 1982), but the convoluted and sometimes karstic geology at the study area may have interacted in an unknown manner with tree abundances to produce the observed pattern. Regardless, the positive effect from trees seems anomalous and suggests that the hydrology of the study area was somewhat unique. The effect of aquifer storage on stream widths was positive, as predicted, but it is not known how accurately

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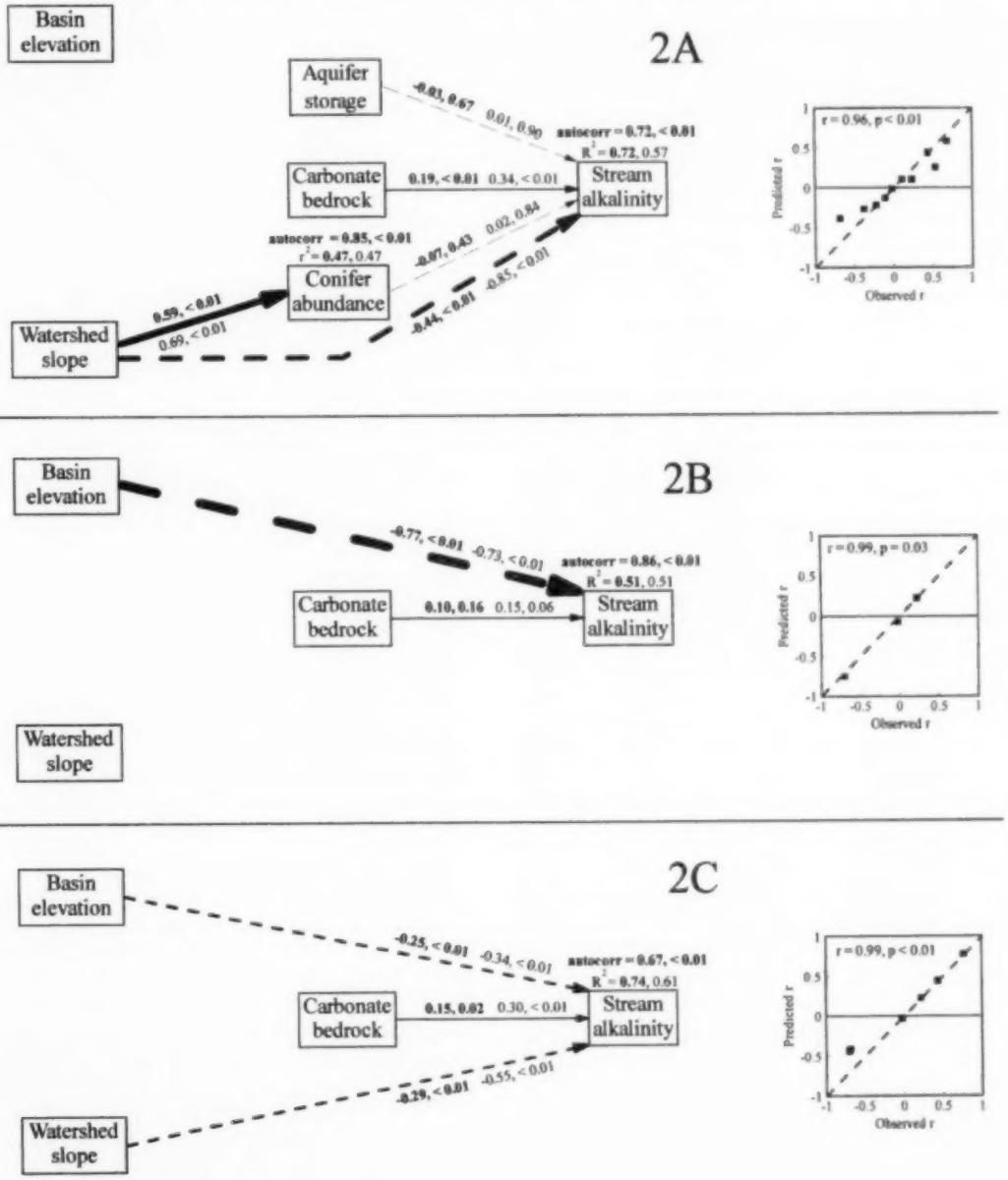
Fig. 4. Path diagrams for three versions of a hypothesis about factors that affect total stream alkalinity of small mountain streams. The width of an arrow is proportional to the strength of an effect that a predictor variable has on a response variable. Numbers next to arrows are standardized partial regression coefficients and probability values derived from spatially explicit regressions (bold type) and conventional least squares regressions. Numbers next to response variables are the autocorrelation coefficients for the spatial regressions and the variation explained by the spatial regressions (bold type) and least squares regressions.



this effect was estimated given that the area of unconsolidated alluvial deposits was used as a surrogate measure. Although alluvium apparently does make contributions to baseflows, a measure that accounted for hydrologic contributions from all geologic formations would have been desirable.

Several factors had no effect on stream width. The absence of a drainage density effect was not expected given the large negative effects previously observed (e.g., Carlston 1963). However, most earlier studies were conducted in the relatively old and mesic mountainous landscapes of eastern North America, where drainage densities are two to seven times greater and probably serve a more important hydrologic role. Road densities were also low relative to other studies (e.g., King and Tennyson 1984; Jones and Grant

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Fig. 5. Path diagram for a hypothesis about factors that affect reach-scale stream slope of small mountain streams. The width of an arrow is proportional to the strength of an effect that a predictor variable has on a response variable. Numbers next to arrows are standardized partial regression coefficients and probability values derived from spatially explicit regressions (bold type) and conventional least squares regressions. Numbers next to response variables are the autocorrelation coefficients for the spatial regressions and the variation explained by the spatial regressions (bold type) and least squares regressions.

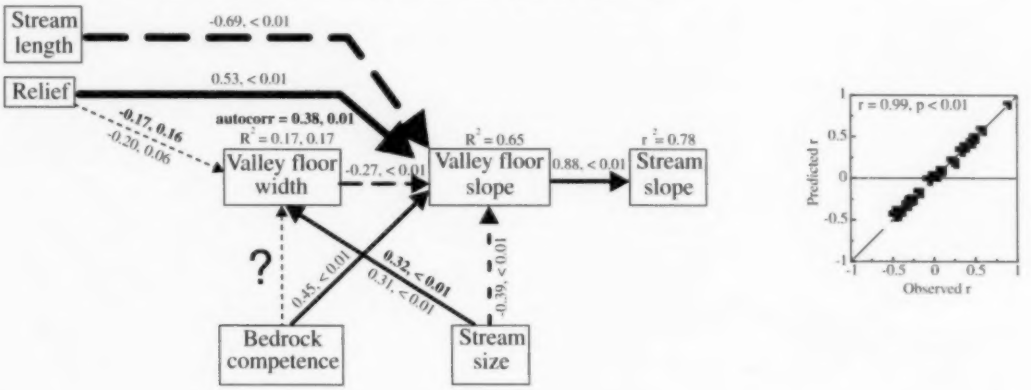

Table 3. Summary of spatial autocorrelation measures and model fits for conventional least squares regressions and spatially explicit regressions.

		Least square regressions				Spatially explicit regressions			
Hypothesis	Response variable	Moran's I	Probability value	R^2	AIC[a]	Autocorrelation coefficient	Probability value	R^2	AIC[a]
1A	STR_WID	0.12	<0.01	0.70	255	0.39	<0.01	0.74	248
	CATTLE	0.42	<0.01	0.29	652	0.93	<0.01	0.28	562
	PAS_GRAS	0.56	<0.01	0.60	−91.4	0.97	<0.01	0.57	−187
	TREE	0.42	<0.01	0.01	−105	0.89	<0.01	0.01	−155
	D_DEN	0.30	<0.01	0.07	20.5	0.78	<0.01	0.05	−6.74
1B	STR_WID	0.08	0.03	0.70	251	0.33	<0.01	0.73	247
	TREE	0.38	<0.01	0.07	−110	0.86	<0.01	0.07	−151
1C	STR_WID	0.12	<0.01	0.70	253	0.39	<0.01	0.73	246
2A	STR_ALK	0.26	<0.01	0.57	863	0.72	<0.01	0.72	836
	CONIFER	0.44	<0.01	0.47	−124	0.85	<0.01	0.47	−171
2B	STR_ALK	0.44	<0.01	0.51	869	0.86	<0.01	0.51	821
2C	STR_ALK	0.31	<0.01	0.61	851	0.67	<0.01	0.74	827
3	STR_SLOP	0.03	0.48	0.78	−13.2				
	VFLR_SLOP	−0.01	0.53	0.65	−58.7				
	VFLR_WID	0.14	0.01	0.17	−687	0.38	0.01	0.17	−692

Note: Spatially explicit regressions were estimated when residuals derived from least squares regressions were not independent. See Table 1 for definitions of variables.

[a]Smaller values indicate better model fits.

1996), which probably explains the negligible effect observed in this study. There was no empirical support for the effect of cattle density on stream width due to changes in channel structure, despite compelling evidence to the contrary from field exclosure experiments (Belsky et al. 1999). This inconsistency, however, may stem from differences in the way studies were conducted. In exclosure studies, the effect of cattle is examined over a limited area and in isolation from all other factors. Conversely, our study attempted to include all of the factors affecting stream width across a broad area and thereby assessed the effect of cattle grazing relative to other factors. When the results of both study types are viewed together, indications are that despite local impacts of cattle grazing, these effects do not translate to landscape-level effects on stream width. Regardless, the combined effects of cattle and sheep on soil compaction do suggest that livestock have some role in determining broad-scale patterns in baseflow widths. This role may be subtler than the direct channel modifications that cattle are typically credited with because it occurs in the uplands and arises from a spatially distributed mechanism, but the effect of soil compaction was second in magnitude only to watershed size. As the effect of soil compaction was also negative, grazing by domestic animals appears to decrease the volume of stream habitat during a period that is critical for stream biota.

Results from the stream alkalinity hypothesis suggest that carbonate bedrock had a positive effect on alkalinities, but this water chemistry attribute was affected most by basin elevation and watershed slope. Unfortunately, collinearity problems stemming from the strong correlation between

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these predictors precluded an accurate assessment of individual effects. Data sets collected in other areas may provide better insight, but the morphology of mountainous watersheds ensures that basin elevation and watershed slope will often be strongly correlated. Stream alkalinity was unaffected by aquifer storage, a result that may be inconclusive given the way aquifer storage was quantified and that most bicarbonate production occurs in the mineral soil layer of hillslopes (Drever 1997). Conifer abundance also had no effect on stream alkalinity, but this relationship is expected to vary among watersheds due to differences in geologic materials.

The causal structure predicted to occur among factors associated with stream slope was largely substantiated by this study. Stream slope had most of its variation explained by the slope of the underlying valley floor, and all of the variables predicted to affect valley floor slope did so. The largest effects on valley floor slope were attributable to stream length and relief, which supports predictions made by earlier researchers (Leopold and Maddock 1953; Cherkauer 1972). Bedrock competence also had a sizeable effect on valley floor slope, despite the crude manner in which competence was quantified. It is unknown how a more accurate measure might affect this parameter estimate, but better resolution would probably provide additional explanatory power.

Spatial autocorrelation

Spatially correlated residuals occurred in most of the least squares regressions that were calculated. Although our sampling design may have contributed to the near ubiquity of autocorrelation within the data, there is no reason to think that other sampling designs could have circumvented this problem and still provided a sufficient sample size and representative correlational structure. In fact, given the tendency for many variables to exhibit gradients or patchiness, violation of the independence assumption is probably a feature common to many linear models based on data generated from a GIS or collected in the field. For the present, however, the frequency with which this assumption is violated will remain unknown until testing for spatially correlated residuals becomes common. Such tests were not easily conducted until recently, but GIS and spatial statistical software (summarized at <www.ai-geostats.org>) now make the detection and incorporation of spatial patterns a routine matter, and these tools should become integral to the development of linear models based on data with a spatial component.

Within the context of this study, spatial autocorrelation was an extraneous consideration that had to be addressed before accurate inference could be drawn. Some anecdotal insights are possible, however, given the frequency with which autocorrelation occurred. Autocorrelation was always positive (i.e., similarity among sites was negatively related to distance), which makes intuitive sense given the variables being modeled, and we suspect that when present, positive autocorrelation will be the norm. Although standard error estimates were not presented, spatial regressions usually provided smaller standard errors than least squares regressions (see Isaak 2001). This result probably occurred because the inclusion of space as a predictor reduced residual variation and facilitated the estimation of more precise parameter estimates for other predictors. Lastly, the amount of change in a parameter estimate between least squares regressions and spatial regressions was directly related to the strength of autocorrelation, indicating that estimation errors were greater when autocorrelation was stronger.

Utility of path analysis

This study was greatly facilitated by path analysis, which despite being closely allied with regression modeling offers several advantages that allow researchers to draw novel inferences. First, many study systems are inherently complex and characterized by chains of causality and interdependence (Christensen et al. 1996). As such, factors that seem to have little direct relevance to a response variable may exert control over that variable through an intermediary. Because conventional regression models have no provisions for dealing with this situation, the result is that such insights are either missed entirely or are erroneously portrayed via a direct effect of a predictor on a response variable. Path analysis, by contrast, has an inherent capacity to deal with this complexity through the interlinkage of regression models. Second, path diagrams present the results of a path analysis in a data-rich, but intuitive format that makes it easy to understand the evolution of a hypothesis, the types of effects that predictors have on response variables, and the relative importance of each predictor. Third, advances in covariance structure analysis (Bollen and Long 1993) have made it possible to compare a predicted data structure with an empirical data structure, thereby providing an assessment of the overall accuracy of a hypothesis and facilitating comparisons among competing hypotheses. Lastly, because path analysis is a causal modeling technique, it requires that a very detailed and very explicit hypothesis be developed before the analysis is conducted. Researchers are thus forced to develop a deep level of familiarity with a study system and to include a plausible mechanism with each pathway included in a hypothesis. This step makes all aspects of the thinking associated with a hypothesis clear and discourages blind data snooping and the weak post hoc explanations that often result.

Despite the opportunities that path analysis offers for a more rigorous application of the scientific method, and that it has become a staple technique in the social science and evolutionary genetics fields (see introductory sections in Klem (1995) and Petraitis et al. (1996)), path analysis has rarely been used within the aquatic sciences. As aquatic systems are not inherently simpler than study systems in other fields, there seems to be no logical reason for this situation. The only limitation of path analysis relative to conventional regression modeling is that a body of knowledge sufficient to develop a comprehensive a priori hypothesis must exist. Whether this requirement is met can only be judged by researchers with expertise in a particular field, but path analysis would seem to be a technique that interested researchers could easily apply given excellent introductory articles (Mitchell 1993; Klem 1995) and widespread familiarity with regression modeling.

Conclusion

We developed and tested hypotheses about how the features of mountain watersheds interact to cause gradients in three stream attributes. By placing broadscale factors with the potential to affect stream attributes into probabilistic

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models, an assessment of the relative importance of each factor across a montane landscape was derived. In all cases, geomorphic features appear to be the primary determinants of habitat gradients, and land surface features were of secondary importance. This work adds a spatial component to the River Continuum Concept that had been lacking and highlights the importance of linkages between terrestrial and aquatic realms. Although our hypotheses would seem to have some generality given the mechanisms involved, these models have only been tested with data from one study area, and caution must be exercised in their interpretation until replicate tests are conducted. Of particular interest would be tests in other Rocky Mountain landscapes where underlying geologies are relatively simple or in other physiographic regions where the ranges of predictor variables might differ markedly. Such tests would yield insights into the mechanisms involved in the production of stream gradients and the spatial domains over which these relationships hold.

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Effects of acclimation to brackish water on the growth, respiratory metabolism, and swimming performance of young-of-the-year Adriatic sturgeon (*Acipenser naccarii*)

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Abstract: Specific growth rates, exercise respirometry, and swimming performance were compared in young-of-the-year Adriatic sturgeon (*Acipenser naccarii*) maintained in freshwater (FW) or acclimated to brackish water (BW) that was slightly hypertonic to sturgeon plasma, at a salinity of 11 g·L^{-1}. Specific growth rate was significantly (17%) lower in BW than in FW. Sturgeon in BW also had a significantly (30%) higher standard metabolic rate than those in FW. In both groups, the relationship between swimming speed and oxygen uptake was described equally well by a linear or exponential equation, with a power relationship between swimming speed and net cost of locomotion and a linear relationship between tailbeat frequency and swimming speed. However, sturgeon in BW exhibited higher mean total oxygen uptake, net costs, and tailbeat frequencies than the FW group at any given swimming speed. There were, however, no differences in aerobic scope or maximum sustainable swimming speed between the FW and BW groups because the BW group exhibited a compensatory increase in active metabolic rate and maximum tailbeat frequency. The results indicate that FW is a more suitable environment than mildly hypertonic BW for young-of-the-year Adriatic sturgeon.

Résumé : Nous avons comparé les taux de croissance spécifiques, la respirométrie à l'exercice et la performance de nage chez des jeunes Esturgeons de l'Adriatique (*Acipenser naccarii*) de l'année gardés en eau douce (ED) ou acclimatés à une eau saumâtre (ES) de salinité de 11 g·L^{-1}, légèrement hypertonique par rapport au plasma d'esturgeon. Le taux de croissance spécifique est significativement plus bas (17%) en ES qu'en ED. Les esturgeons en ES ont aussi un taux métabolique standard significativement plus élevé (30%) que les esturgeons en ED. Chez les deux groupes, la relation entre la vitesse de nage et la consommation d'oxygène se décrit aussi bien par une équation linéaire que par une équation exponentielle, avec une relation exponentielle entre la vitesse de nage et le coût net de la locomotion et une relation linéaire entre la fréquence de battement de la queue et la vitesse de nage. Cependant, quelle que soit vitesse de nage, les esturgeons en ES ont une consommation d'oxygène moyen total, des coûts nets et des fréquences de battements de la queue plus élevés que ceux qui sont en ED. Il n'y a toutefois aucune différence dans l'amplitude aérobique ou la vitesse de nage maximale maintenue entre les deux groupes parce que les esturgeons en ES font montre d'une augmentation compensatoire de leur taux de métabolisme actif et de leur taux de battement de la queue. Il en résulte que, pour les jeunes Esturgeons de l'Adriatique de l'année, l'ED est un milieu plus approprié que l'ES légèrement hypertonique.

[Traduit par la Rédaction]

Introduction

The life cycle of many sturgeon species includes repeated migrations between freshwater (FW) and estuarine or marine habitats (Rochard et al. 1991; Birstein 1993). The Adriatic or Italian sturgeon (*Acipenser naccarii*) exhibits a diadromous life history: breeding occurs in FW, both juveniles and adults are reported to inhabit brackish estuarine waters (Rossi et al. 1992), and adults have been captured in the Adriatic Sea (Tortonese 1989). There are also anecdotal reports of egg deposition in brackish estuarine waters in this species (Paccagnella 1948).

There are very few published studies on the osmoregulatory physiology of acipenserids (e.g., Potts and Rudy

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1972; Natochin et al. 1985; Krayushkina et al. 1996). A preliminary study on *A. naccarii* by Cataldi et al. (1995) revealed that 1- to 1.5-year-olds were able to tolerate brackish water (BW) at a salinity of 20 $g \cdot L^{-1}$ and seawater (SW) at 30 $g \cdot L^{-1}$ for 60 days and regulate plasma osmolality and ions at levels similar to those in sturgeon kept in FW. Nonetheless, there was some evidence of difficulty in long-term adaptation, particularly at 30 $g \cdot L^{-1}$, with plasma osmolality gradually increasing over time (Cataldi et al. 1995). Subsequent studies have revealed that young-of-the-year (YOY) sturgeon acquire the ability to tolerate hyperosmotic environments once they have completed development of the gills and kidney, but the 96-h LC50 for salinity in such sturgeon (aged 120 days) was approximately 23 $g \cdot L^{-1}$ (Cataldi et al. 1999). Following a 42-day acclimation to BW at 11 and 23 $g \cdot L^{-1}$, slightly older animals (aged 150 days) were able to regulate plasma osmolality and ion concentrations at levels similar to those of their siblings in FW, as a consequence of adaptive responses that included an increase in the activity of Na^+,K^+-ATPase in the gills (McKenzie et al. 1999).

The effect of salinity on the growth and metabolic rate of euryhaline teleosts (particularly salmonids) has been the focus of many studies (for a review, see Morgan and Iwama 1991). Although early papers indicated that growth might be promoted in BW at an osmolality similar to that of the plasma, as a consequence of reduced metabolic costs for osmoregulation (Canagataram 1959; Rao 1968), recent theoretical and experimental studies (Kirschner 1995; Morgan and Iwama 1999) indicate that osmoregulatory costs are quite low in teleosts and that growth rates are highest and metabolic rates lowest in the salinity that is appropriate for the life stage of the species under study (Morgan and Iwama 1991). An investigation of the effects of salinity on growth rates of euryhaline chondrosts may provide information about the optimal environment for animals of a particular age and size.

A previous study compared the growth of YOY sturgeon in FW with that in BW at a salinity of 20 $g \cdot L^{-1}$ and osmolality of approximately 565 $mosmol \cdot kg^{-1}$ (McKenzie et al. 1999). Sturgeon plasma has an osmolality of approximately 280 $mosmol \cdot kg^{-1}$, so BW at an osmolality of 565 $mosmol \cdot kg^{-1}$ was as hypertonic to sturgeon plasma as the FW was hypotonic. Therefore, costs for osmoregulation, whatever their absolute magnitude, should have been similar in the two environments (Kirschner 1995; Morgan and Iwama 1999). The BW at 20 $g \cdot L^{-1}$ caused a profound inhibition of growth (McKenzie et al. 1999), indicating that YOY sturgeon were able to tolerate a requirement for hyperosmoregulation much better than an equal but opposite requirement for hypoosmoregulation.

In the wild, migrating sturgeon are known to spend a period in BW prior to moving into the marine environment, and this is particularly true for juveniles, for which the period can last for many months or years (Sulak and Clugston 1999). This may indicate that time spent in BW promotes hypoosmoregulatory adaptations necessary for subsequent tolerance of SW but, presumably, only if the water were hypertonic to sturgeon plasma. The current paper investigated the effects of acclimation to slightly hypertonic BW on growth rates of YOY Adriatic sturgeon, employing water at a salinity of 11 $g \cdot L^{-1}$ and osmolality of 310 $mosmol \cdot kg^{-1}$. The current study is linked to a companion paper that investigated whether such prior acclimation to a mildly hypertonic environment promoted tolerance of a further salinity challenge (McKenzie et al. 2001).

Swimming respirometry has been used extensively to describe aspects of respiratory metabolism in teleosts (Brett 1964; Fry 1971; Beamish 1978), including estimates of standard metabolic rate (SMR) (Brett 1964; Fry 1971) that might reveal metabolic costs associated with salinity acclimation. Swimming performance is also an integrated index of fitness in fish, and differences in performance have been used to reveal underlying stresses associated with nonoptimal abiotic factors such as salinity (Randall and Brauner 1991) or sublethal exposure to toxicants (Beaumont et al. 1995). Swimming performance of acipenserids has been studied very little (Webb 1986; McKinley 1991; Peake et al. 1997) but is of considerable interest because of the migratory lifestyle of many sturgeon species. In the current study, the growth rates of sturgeon in FW or BW at a salinity of 11 $g \cdot L^{-1}$ were compared with measures of exercise-related respiratory metabolism and swimming performance to reveal any metabolic costs and consequences for performance of acclimation to mildly hypertonic BW as compared with FW.

Materials and methods

Experimental animals

Fertilised eggs of *A. naccarii* were obtained from the artificial reproduction of captive broodstock (Arlati et al. 1988) at the Azienda Agricola VIP di Giovannini Giacinto (Orzinuovi (BS), Italy) in July 1997. Eggs were transported to the "La Casella" Experimental Thermal Aquaculture Plant (Sarmato (PC), Italy) and hatched indoors on grid incubators provided with a flow of aerated well water at 15°C. The water had the following ionic composition (millimolar) and characteristics: Ca^{2+} = 2.3, Mg^{2+} = 0.6, Na^+ = 2.3, Cl^- = 0.7, K^+ = 0.9, titratable alkalinity to pH 4 = 13.5 mM, total hardness = 240 $mg \cdot L^{-1}$ as $CaCO_3$, and pH 8.2. First-feeding of the fry was with *Artemia* nauplii, followed by gradual adaptation to artificial granulated dry feed (Trouvit Storioni, Hendrix SpA, Mozzecane (VR), Italy). Photoperiod was natural via skylights.

The fry were subsequently transferred to 1-m^2 fiberglass tanks (water volume approximately 250 L) and gradually adapted to a flow of aerated FW at a temperature of 23 ± 1°C within a closed-cycle hatchery biofilter (total volume 90 m^3), with temperature raised over a 16-day period by proportional mixing of well water and biofilter water at the inflow. The biofilter water had the same chemical characteristics as the well water, from which it was derived, but with a water pH of 8.1. Sturgeon were weighed at 30-day intervals and feeding rate maintained at approximately 2% of mean mass per day, using feed (Trouvit Storioni) of increasing granule size, as required.

In December 1997, the sturgeon had achieved a mean (±SE) mass of 14.2 ± 0.7 g and mean fork length of 11.8 ± 0.2 cm (N = 173). It has previously been established that Adriatic sturgeon of this size and age can tolerate salinities up to 23 $g \cdot L^{-1}$ (Cataldi et al. 1999; McKenzie et al. 1999). Six groups of 15 animals, selected at random and with no differences in resultant mean mass, were transferred into 4-m^2 fiberglass tanks (water volume approximately 1000 L). Three of the tanks continued to receive a flow of aerated FW from the closed-cycle biofilter. The remaining three tanks received a flow of aerated BW at a salinity of 11 $g \cdot L^{-1}$ and temperature of 23 ± 1°C, provided from a closed-cycle biofilter with a total volume of approximately 6 m^3. The BW was composed of well water to which marine salts (ProdacMare, Prodac, Citadella (PD),

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Italy) were added and had a pH of 8.4. These triplicate groups of sturgeon in FW and BW were the subjects of the growth study.

Two further 4-m^2 fiberglass tanks were also prepared containing 25 randomly selected animals, one of which received a flow of FW and the other a flow of BW. These tanks provided subjects for the swimming respirometry experiments and the analyses of haematological variables in FW versus BW.

All tanks received a similar water flow rate of approximately 5 $L \cdot s^{-1}$. The sturgeon were allowed approximately 70 days (10 weeks) to acclimate to their respective salinities, with total tank mass measured every 30 days to adjust feeding rates. Feed was provided at 3% wet body mass per day.

At the beginning of March 1998, the groups of sturgeon acclimated to FW and BW had achieved a mean mass of approximately 100 g and fork length of 23 cm, a size at which they could be tagged for individual identification. They were anaesthetized (100 mg tricaine methanesulphonate$\cdot L^{-1}$) and tagged with colored glass beads threaded onto sterilized nylon fishing line sutured to the dorsal fin. All animals recovered within 5 min of replacement into aerated water in their tanks; there was no mortality associated with the procedure.

Growth

The effects of water salinity on growth were measured on the acclimated animals over a 63-day (9-week) period, during which the sturgeon were fed daily by belt feeder over a 2-h period starting at 17:00 with a ration equivalent to 3% wet body mass. Mass and total fork length of the triplicate groups of tagged individuals in FW and BW were measured at 21-day (3-week) intervals. Cumulative specific growth rate (SGR) was calculated at each interval as percent increase in mass per day. Condition factor (CF) was calculated as $100 \times (\text{mass}/\text{length}^3)$.

Swimming respirometry

The effects of acclimation to FW or BW on respiratory metabolism and exercise performance were investigated with a Brett-type swim-tunnel respirometer constructed in polyvinyl chloride, acetal, and acrylic material. A circulating flow of water was generated in the tunnel (total volume 49 L) by an acrylic propeller attached to a variable-speed DC permanent magnet motor (Brook Hansen MP 80 115 DH, Bell Electric, Birmingham, U.K.). The voltage delivered to the motor was controlled by a personal computer and Labview software (National Instruments, Austin, Tex.) via a data acquisition interface board (CB 68LP, National Instruments). The voltage was calibrated within Labview in order to deliver controlled water velocities between 10 and 90 $cm \cdot s^{-1}$. Sturgeon were placed downstream of the propeller in a swimming chamber with a square 225-cm^2 cross-sectional area. Vanes were positioned between the propeller and swimming chamber to provide nonturbulent water flow and uniform water velocity across the entire section of the swimming chamber (Steffensen et al. 1984).

When sealed, the respirometer permitted measurement of oxygen uptake ($\dot{M}O_2$) by the sturgeon. A small fraction of the water from the sealed respirometer was siphoned past a Clarke-type polarographic oxygen electrode (model E5041, Radiometer, Copenhagen, Denmark) in a cuvette with the thermostat set to 25°C with a water bath (RCS200, MGW Lauda, Königshofen, Germany). An oxygen meter (model 781, Strathkelvin Instruments, Glasgow, Scotland) displayed the $P_{W_{O_2}}$ in the respirometer, with the signals recorded by the personal computer and Labview software via the interface board. Information on changes in $P_{W_{O_2}}$ over time during the period of closed-cycle circulation was stored as text files, and then linear regressions between time and $P_{W_{O_2}}$ were calculated with a spreadsheet program (Excel 97, Microsoft). The resultant slopes were used to quantify oxygen consumption with appropriate values for fish mass, respirometer water volume, and oxygen solubility in water at the experimental temperature and salinity. Only slopes with $r^2 > 0.9$ were used in the calculations.

The respirometer chamber was immersed in a large tank of aerated water. When the $P_{W_{O_2}}$ in the respirometer chamber declined below 90% saturation, the Labview software activated a flushing pump (1048UHP, Eheim, Germany) that flushed aerated water from the outer tank through the respirometer chamber, bringing the $P_{W_{O_2}}$ back to 95% saturation, at which point the pump switched off and measurement of oxygen consumption resumed.

At the end of each experiment, blank tests were run to measure the proportion of total $\dot{M}O_2$ that was attributable to bacterial metabolism. The measured rates in the absence of a fish never exceeded 5% of the total $\dot{M}O_2$ and in most cases were negligible. As such, no corrections were applied. The oxygen electrode was calibrated at the beginning and the end of each experiment; zero current was set by unplugging the electrode from the meter and air saturation was then set by reconnecting the electrode and exposing it to a flow of 100% air-saturated water.

Individual sturgeon that had previously been starved for at least 24 h were placed in the respirometer containing water at the appropriate acclimation salinity and forced to swim at a water speed equal to 0.5 bodylength $(BL) \cdot s^{-1}$ for at least 12 h (overnight). This swimming speed was chosen because it is similar to the speed of spontaneous activity in this species (McKenzie et al. 1995). The following day, the fish were exposed to progressive increments in swimming speed of 0.5 $BL \cdot s^{-1}$ every 60 min until fatigue. Fish were considered to be fatigued when they were unable to remove themselves from the posterior screen of the swimming chamber despite gentle repeated prodding.

Measurements of $\dot{M}O_2$ were collected at each swimming speed. For each fish, a least squares exponential regression was applied to the relationship between swimming speed and $\dot{M}O_2$. The y-intercept of the regression equation was taken as the theoretical rate of $\dot{M}O_2$ of the stationary fish (Brett 1964). This value is considered to be an indicator of metabolic rate in the notional absence of locomotor muscle activity, and hence of maintenance metabolism or SMR (Brett 1964; Fry 1971). Active metabolic rate (AMR) was taken as the maximum $\dot{M}O_2$ measured during exercise in the last increment entirely completed before fatigue (Fry 1971; Webb 1993). SMR was subtracted from AMR to estimate net aerobic scope (Fry 1971). The value of SMR was also subtracted from the $\dot{M}O_2$ for each swimming speed to reveal the sturgeon's net cost of locomotion (Beamish 1978). Mean rates of total and net $\dot{M}O_2$ were also calculated at each speed for comparison between the two groups.

Fish were filmed at each swimming speed (Video 8) to allow counts of tailbeat frequency and gill ventilation frequency. Tailbeat frequency at each speed was used as an index of propulsive muscular power in the two groups, while analysis of ventilation frequency provided insight into the extent to which increases in $\dot{M}O_2$ were linked to increased ventilatory drive and whether the sturgeon exhibited a transition to ram ventilation during swimming. Mean tailbeat and ventilation frequencies were also calculated for each swimming speed.

Maximum sustainable (critical) swimming speed (U_{crit}, body lengths per second) was calculated using the equation provided by Brett (1964), which adds the velocity of the most recently completed increment to the product of the incremental increase in velocity (i.e., 0.5 $BL \cdot s^{-1}$) and the proportion of the final increment completed before fatigue. No correction for the solid blocking effect of the fish was considered, as the total cross-sectional area of the fish did not exceed 5% of that of the swimming chamber (Bell and Terhune 1970).

Haematological variables

To investigate characteristics of osmoregulatory homeostasis in FW versus BW, blood samples (1 mL) were collected from the caudal vein of sturgeon acclimated to the two salinities and plasma

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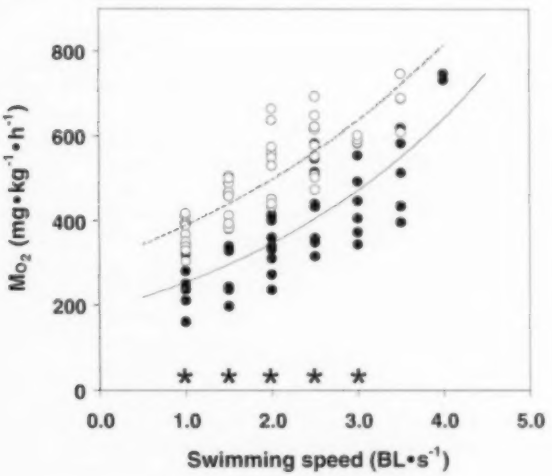
Table 1. Mean (±SE) mass, length, condition factor (CF), and cumulative SGR in Adriatic sturgeon maintained in either FW or BW at a salinity of 11 g·L^{-1}.

		Day 0	Day 21	Day 42	Day 63
Mass (g)	FW	94±6	131±8	206±12	279±17
	BW	112±10	163±12	217±16	290±19
Length (cm)	FW	22.6±0.6	25.1±0.6	28.5±0.6	31.9±0.6
	BW	23.0±0.7	26.4±0.7	28.9±0.8	32.5±0.7
CF (100 g·cm^{-3})	FW	0.79±0.02	0.79±0.01	0.85±0.01	0.82±0.01
	BW	0.86±0.01*	0.83±0.01*	0.85±0.02	0.81±0.02
SGR (%·day^{-1})	FW		1.64±0.07	2.72±0.12	2.96±0.11
	BW		2.07±0.15*	2.26±0.17*	2.47±0.20*

Note: N = 38 for FW and N = 28 for BW. *Significantly different from the FW group at that time (t test, $P < 0.05$).

Fig. 1. Relationship between swimming speed and $\dot{M}O_2$ in Adriatic sturgeon acclimated to FW (solid circles) or BW at a salinity of 11 g·L^{-1} (open circles). For FW, the solid line describes the exponential relationship $y = 187e^{0.308x}$ ($R^2 = 0.625$, $N = 47$ observations on eight fish). For BW, the broken line describes the exponential relationship $y = 303e^{0.247x}$ ($R^2 = 0.699$, $N = 43$ observations on nine fish). The lines are also described by the linear equations $y = 124x + 118$ ($R^2 = 0.636$) and $y = 122x + 265$ ($R^2 = 0.701$) for FW and BW, respectively. The asterisks on the abscissa indicate those swimming speeds at which BW sturgeon exhibited a significantly higher mean $\dot{M}O_2$ than FW fish (t test, $P < 0.05$).



osmolality, Na^+, Cl^-, and K^+ measured. Plasma lactate was also measured to investigate the effects of exercise on anaerobic metabolism.

For control samples on fish that had not been exercised, care was taken to avoid any exposure of the sturgeon to air prior to sampling under anaesthesia. Individual fish were gently netted into a bucket underwater, the bucket withdrawn from the tank, 100 mg tricaine methanesulphonate·L^{-1} added, and the fish then sampled immediately following loss of ventilatory movements. This protocol was followed to ameliorate any confounding effects of acute sampling stress on plasma osmolality and ion concentrations (Di Marco et al. 1999). For animals swum to fatigue, caudal samples were collected immediately following fatigue while the fish were resting passively in the swimming respirometer.

Plasma was separated by centrifugation. Plasma osmolality was measured with an osmometer (Fiske One-Ten, Fiske Associates, Needham Heights, Mass.); Na^+, K^+, and Cl^- were measured by electrolyte analyser with ion-specific electrodes (SPOTCHEM SE 1510, Menarini, Milan, Italy). Plasma samples were frozen and stored in liquid nitrogen for a maximum of 2 weeks prior to thawing and analysis of lactate with an analytical kit (Sigma Lactate 826B).

Following collection of blood samples (and swimming respirometry, as appropriate), the tagged fish were returned to their holding tanks in either FW or BW. There were no mortalities associated with the sampling procedure or swimming experiments.

Statistical analysis

All statistical analyses were performed with the Sigmastat software package (Jandel Scientific, San Rafael, Calif.). Within the FW or BW group, the changes in meristic variables over time were assessed by one-way analysis of variance (ANOVA) for repeated samples, with a Bonferroni multiple comparisons t test to identify significant differences among means. For any given measurement interval, meristic variables were compared between the FW and BW groups by t test. To describe the relationships between respirometric or performance variables during the exercise protocol, linear, exponential, or power functions were applied and the function with the highest correlation coefficient identified. For any given measurement interval during the exercise protocol, variables were compared between the FW and BW groups by t test. All single variables (e.g., U_{crit}) were also compared between the two groups by t test. In all cases, $P < 0.05$ was taken as the fiducial level of significance.

Results

During the 70-day period of acclimation to their respective salinities, prior to tagging of the sturgeon and commencement of the growth study, the three tanks of sturgeon in FW exhibited a mean (±SE) cumulative mortality of 15.5 ± 2.2%, significantly lower than the mortality of 37.7 ± 2.3% observed in BW. The mortality was not due to disease but can occur during the early life stages of this species and is presumably a consequence of the paucity of information on optimal husbandry requirements. In particular, the mortality is believed to have been a result of interacting factors related to fry quality following artificial reproduction and the acceptance by fry of artificial feeds. The sturgeon that survive these delicate early stages are extremely robust, and there has been no mortality directly attributable to disease in the populations of *A. naccarii* currently maintained at La Casella.

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Table 2. Mean (±SE) values for selected exercise-related metabolic and performance variables in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1}.

	FW (N = 8)	BW (N = 9)
SMR (mg O_2·kg^{-1}·h^{-1})	216±25	281±11*
AMR (mg O_2·kg^{-1}·h^{-1})	548±61	635±22
Aerobic scope (mg O_2·kg^{-1}·h^{-1})	332±47	354±20
Maximum tailbeat frequency (Hz)	3.91±0.14	4.49±0.17*
Maximum ventilation rate (beats·min^{-1})	127±4	146±4*
U_{crit} (BL·s^{-1})	3.23±0.24	2.75±0.22

Note: *Significantly different from the FW group (t test, $P < 0.05$).

Fig. 2. Relationship between swimming speed and net cost of locomotion (as M_{O_2}) in Adriatic sturgeon acclimated to FW (solid circles) or to BW at a salinity of 11 g·L^{-1} (open circles). For FW, the solid line describes the power relationship $y = 45.8x^{1.44}$ (R^2 = 0.656, N = 47 observations on eight fish). For BW, the broken line describes the power relationship $y = 93.8x^{1.22}$ (R^2 = 0.814, N = 43 observations on nine fish). The asterisks on the abscissa indicate those swimming speeds at which BW sturgeon exhibited a significantly higher mean net cost than FW fish (t test, $P < 0.05$).

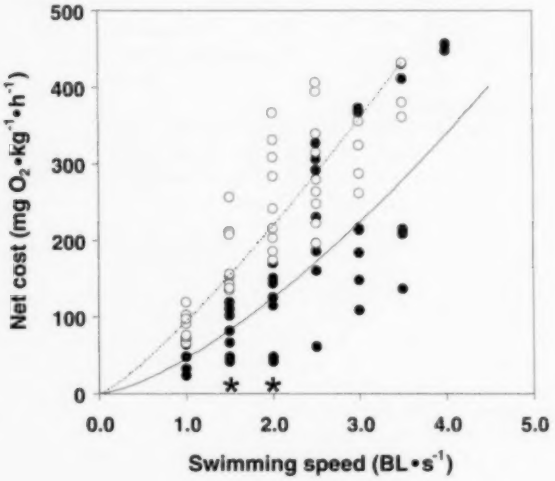
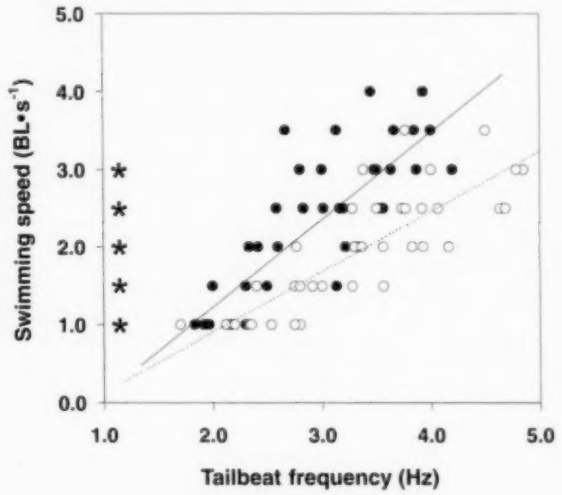


Fig. 3. Relationship between tailbeat frequency and swimming speed in Adriatic sturgeon acclimated to FW (solid circles) or to BW at a salinity of 11 g·L^{-1} (open circles). For FW, the solid line describes the linear relationship $y = 1.123x - 1.024$ (R^2 = 0.646, N = 49 observations on eight fish). For BW, the broken line describes the linear relationship $y = 0.776x - 0.646$ (R^2 = 0.714, N = 45 observations on nine fish). The asterisks on the ordinate indicate those swimming speeds at which BW sturgeon exhibited a significantly higher mean tailbeat frequency than FW fish (t test, $P < 0.05$).



Growth

At the beginning of the growth study, there were no differences in mean mass, length, or CF among the three FW groups or among the three BW groups. The tagged animals were therefore pooled to represent one group for each salinity. The resultant FW and BW groups had the grand means for mass, length, and CF presented in Table 1. There were no differences in mass or length between fish at the two salinities, but the sturgeon in FW had a significantly lower CF.

During the 63-day growth experiment, there were three mortalities in one BW tank and one mortality in each of two FW tanks; mean mortality rate did not differ, therefore, between the salinities. Results reported below only consider those animals that survived the entire growth experiment. Within each salinity group, there were no differences between the three replicate tanks for mean mass, SGR, or CF at any measurement interval during the 63-day growth experiment. Thus, analysis has been restricted to a comparison of the grand means of all individual FW sturgeon versus all individual BW animals (Table 1). Both groups exhibited significant increases in mass and length; mean mass and length were very similar between the two groups with no significant differences at any measurement interval (Table 1). SGRs were high in both groups, ranging between 1.6 and almost 3%·day^{-1} (Table 1). At a feeding rate of 3% body mass per day, this represents a feed conversion (i.e., ratio of feed provided to weight gain) of between 0.5 and 1. There were, however, some differences in SGR between the groups, whereby the BW sturgeon grew significantly better than their FW counterparts over the first 21 days, but by 42 days, the FW group exhibited a significantly better growth rate and, when the entire 63-day period was considered, the FW group exhibited a significantly higher overall SGR than the BW fish (Table 1). The difference in CF between FW and BW observed at the beginning of the growth experiment had disappeared by 42 days and, at that time

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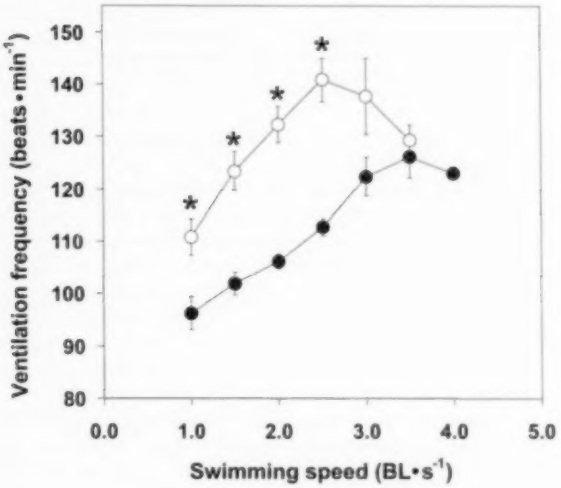
Fig. 4. Mean (±SE) gill ventilation rates at increasing swimming speeds in Adriatic sturgeon acclimated to FW (solid circles) or to BW at a salinity of 11 g·L^{-1} (open circles). For FW, $N = 8$ from 1 to 3 BL·s^{-1}, $N = 5$ at 3.5 BL·s^{-1}, and $N = 3$ at 4 BL·s^{-1}. For BW, $N = 9$ from 1 to 2.5 BL·s^{-1}, $N = 4$ at 3 BL·s^{-1}, and $N = 3$ at 3.5 BL·s^{-1}. The asterisks denote those swimming speeds at which the BW fish had higher ventilation frequencies than FW fish (t test, $P < 0.05$).



interval and at 63 days, there were no differences in CF among the two salinities (Table 1).

Swimming respirometry

Effects of salinity acclimation on exercise performance were studied on eight animals from the FW group and nine from the BW group. These had mean (±SE) weights and lengths of 156 ± 12 g and 26.3 ± 0.4 cm and 158 ± 16 g and 26.9 ± 1.0 cm in the FW and BW groups, respectively, with no significant differences between the groups.

Stepwise increases in swimming speed elicited an increase in $\dot{M}O_2$ by the sturgeon from both FW and BW (Fig. 1), with the resultant relationships described almost equally well by a linear or an exponential function. Note that $\dot{M}O_2$ measured at 0.5 BL·s^{-1} is not included in the analyses, as data were highly variable at that speed due to differences in spontaneous activity between the sturgeon, which disappeared when current velocity rose to 1 BL·s^{-1} (Webb 1993). As is clearly visible in Fig. 1, however, there were marked differences in $\dot{M}O_2$ between the groups, with the BW group showing a curve that was shifted towards higher rates of $\dot{M}O_2$ at any given swimming speed. Indeed, a comparison between the two groups of mean $\dot{M}O_2$ at each swimming speed revealed that the fish in BW consumed significantly more oxygen than did those in FW when swimming at all speeds between 1 and 3 BL·s^{-1} inclusive. Calculation of SMR (Brett 1964) revealed that sturgeon acclimated to BW had a significantly higher SMR than those maintained in FW (Table 2), with an increase of about 30%. AMR was 15% higher in the BW group than in the FW group, but the difference was not statistically significant ($P = 0.09$) and there were no differences in aerobic scope between the two salinities (Table 2).

Figure 2 shows the net cost of locomotion (as $\dot{M}O_2$) of sturgeon in FW or BW. The relationship between swimming speed and cost of locomotion was best described by a power function in both groups. However, the relationship described by the sturgeon acclimated to BW was shifted noticeably upwards relative to that of the sturgeon maintained in FW, revealing higher net costs for the BW fish when swimming at any given speed. A comparison of mean costs at each speed revealed these to be significantly higher in the BW group when swimming at speeds of 1.5 and 2.0 BL·s^{-1}.

Figure 3 shows the relationship between tailbeat frequency and swimming speed in the sturgeon acclimated to the two different salinities. The relationship was best described by a linear function in both FW and BW. As is visible in Fig. 3, however, the relationship described by the sturgeon acclimated to BW is shifted to the right when compared with the relationship for the fish in FW, such that BW sturgeon required higher tailbeat frequencies to achieve any given swimming speed. The mean frequencies were significantly higher in BW fish at all swimming speeds between 1 and 3 BL·s^{-1} inclusive. The BW group also had a significantly higher maximum tailbeat frequency than that measured in the FW group (Table 2).

In both experimental groups, stepwise increases in swimming speed were linked to a significant hyperventilation (Fig. 4). In the FW group, there was a linear increase in ventilation rate with increased swimming speed (Fig. 4). When swimming at low to intermediate speeds, the sturgeon acclimated to BW exhibited significantly higher mean ventilation rates than those in FW (Fig. 4). At the highest speeds, the fish in BW exhibited a decline in ventilatory rate that reflected a partial shift towards ram ventilation (Fig. 4). The change towards ram ventilation at high swimming speeds was, however, only partial, as ventilatory rate remained elevated if compared with the rates measured at the lowest swimming speeds (Fig. 4). The animals of the BW group exhibited a significantly higher maximum ventilation rate than the sturgeon in the FW group (Table 2).

Critical swimming speeds for the sturgeon acclimated to the two salinities are presented in Table 2. Although mean U_{crit} was 17% higher in FW than in BW, the difference was not statistically significant.

Haematological variables

Effects of salinity acclimation on haematological variables in sturgeon without exercise (i.e., under resting conditions) were studied on six animals from each of the FW and BW groups. These had mean (±SE) masses of 167 ± 15 and 163 ± 17 g in the FW and BW groups, respectively, with no significant difference between the groups. Plasma osmolality and ion concentrations are presented for the BW and FW sturgeon in Table 3. Plasma osmolality was equal in both groups, but the sturgeon acclimated to BW had slightly but significantly higher plasma Na^+ and Cl^- concentrations than those in FW.

Plasma osmolality in the FW and BW sturgeon following exercise to fatigue was not significantly different from the values measured in resting fish from the same group, but because mean osmolality decreased in FW and increased in BW, osmolality was significantly higher in the BW sturgeon postexercise as compared with the FW fish (Table 3). Exer-

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Table 3. Mean (±SE) plasma osmolality, ion concentrations, and lactate concentration in Adriatic sturgeon acclimated to either FW or BW at 11 g·L^{-1} at rest and following exercise to fatigue.

	FW		BW	
	Rest (N = 6)	Fatigue (N = 8)	Rest (N = 6)	Fatigue (N = 9)
Osmolality (mosmol·kg^{-1})	277±3	267±7	278±5	302±10*
Na^+ (mequiv.·L^{-1})	138±1	138±2	146±2*	151±2*#
Cl^- (mequiv.·L^{-1})	115±2	117±4	133±4*	139±4*†
K^+ (mequiv.·L^{-1})	4.0±0.2	3.7±0.2	3.8±0.2	4.1±0.2
Lactate (mmol·L^{-1})	0.58±0.05	1.41±0.63#	0.70±0.03	1.58±0.48#

Note: *Significantly different from the FW group under the same conditions; #significantly different from the resting value in the same group; †significantly different from the resting value in the FW group (one-way ANOVA, $P < 0.05$).

cise caused a significant increase in plasma Na^+ and Cl^- levels in BW sturgeon but not in FW fish. As a consequence, the exercise exacerbated the differences in Na^+ and Cl^- concentrations between the two groups (Table 3). There were no differences in plasma lactate among the groups under resting conditions, and exercise caused a significant increase in plasma lactate concentrations in both groups (Table 3).

Discussion

The poorer survival and growth rates of the YOY sturgeon acclimated to a water salinity of 11 g·L^{-1} as compared with their siblings maintained in FW clearly indicate that mildly hypertonic BW is a less optimal environment than FW for sturgeon of that age and (or) size. A previous growth study on this species at approximately the same age found a marked inhibition of growth in sturgeon acclimated for 2 months to BW at 20 g·L^{-1}, SGR fell by 56% relative to siblings maintained in FW, and the animals in BW exhibited 100% mortality following the use of heavy machinery near their tanks (McKenzie et al. 1999). The current study found a 17% inhibition of SGR in BW at 11 g·L^{-1}, and mortality in BW was over twice that in FW.

The reduced growth rate in BW can be attributed to the increased SMR and therefore energetic costs for maintenance metabolism in that environment. The increased SMR may represent the metabolic cost of osmoregulation. There are, however, a number of reasons why this is unlikely to have been the case. The osmolality of the BW in the present study (approximately 310 mosmol·kg^{-1}) only exceeded that of sturgeon plasma by about 10%, so the costs of osmoregulation should in fact have been reduced in the BW relative to FW. YOY sturgeon acclimated for 6 weeks to BW at 11 g·L^{-1} do not exhibit a significant difference from their FW siblings with regard to the activity of the Na^+,K^+-ATPase in crude gill homogenates (McKenzie et al. 1999). Furthermore, in teleosts, the costs of osmoregulation may in fact be very low (Kirschner 1995; Morgan and Iwama 1999), and rates of oxygen consumption do not always reflect expected costs of osmoregulation (Morgan and Iwama 1998). Indeed, although early studies reported lower metabolic rates and improved growth in salmonids acclimated to BW at an osmolality approaching that of their body fluids (Canagaratnam 1959; Rao 1968), the consensus to emerge from more recent studies is that salmonid fry and parr will exhibit elevated metabolic rate and poor growth in salinities other than the natural one typical for their life stage at that time, even in salinities almost isotonic with their plasma (Morgan and Iwama 1991).

Morgan and Iwama (1991, 1998, 1999) argued that the increased SMR and poor growth that are observed in juvenile salmonids maintained at inappropriate salinities reflect influences on metabolism other than metabolic costs for fluid and ion homeostasis. For example, chronic stress-related activation of the hypothalamic–pituitary axis is known to raise metabolic rate and inhibit growth in teleosts (Wendelaar Bonga 1997). Although the stress of handling and anaesthesia caused a marked increase in plasma cortisol in adult Adriatic sturgeon (Di Marco et al. 1999), YOY sturgeon acclimated for 6 weeks to salinities of 11 and 23 g·L^{-1} exhibited no differences in plasma cortisol concentrations relative to their siblings in FW (McKenzie et al. 1999). Thus, the increased metabolic rate and reduced growth of the sturgeon in BW may reflect other effects of chronic sublethal stress.

Morgan and Iwama (1991) reviewed the literature regarding the effects of salinity on metabolic rate in euryhaline teleosts and proposed that they be grouped into five classes. Class III includes the presmolt stages of anadromous salmonids, for whom metabolic rate is lowest in FW and increases in waters of higher salinities (Morgan and Iwama 1991). The results of the current study would seem to place YOY Adriatic sturgeon in the same class, as BW at a salinity of 11 g·L^{-1} elicited a significant increase in SMR and inhibition of growth.

The estimation of maximum sustainable aerobic swimming speed for the Adriatic sturgeon is the first such report for a chondrost and acipenserid. Critical swimming speed in FW was estimated to be about 15% lower than that of sockeye salmon (*Oncorhynchus nerka*) of similar length (Brett 1965). Webb (1986) reported that lake sturgeon (*Acipenser fulvescens*) exhibits swimming kinematics that are similar to those of teleosts but that the thrust generated at cruising speeds is 18% less than in similar-sized trout, presumably as a consequence of the heterocercal acipenserid caudal fin. The lake sturgeon's capacity for burst exercise, namely exercise fueled by anaerobic processes for short periods at high speeds (Beamish 1978), is significantly inferior to that of similar-sized salmonids (Peake et al. 1997).

The shallow relationship between swimming speed and log $\dot{M}O_2$ in both FW and BW may indicate that sustained aerobic exercise in sturgeon is energetically quite efficient (Beamish 1978). McKinley (1991) reported that the relationship between swimming speed and $\dot{M}O_2$ was best described by a linear equation in lake sturgeon. Although an exponential relationship should be expected for energetic reasons

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(Fry 1971; Beamish 1978), this is not necessarily the case for all teleost species (Webb 1993). Nonetheless, when the net cost of locomotion was calculated, it was best described by a power function, which is the expected energetic relationship (Beamish 1978). If confirmed in future studies, the apparent energetic efficiency of sustained aerobic swimming in sturgeon would be of obvious adaptive advantage to species that perform repetitive long annual migrations between FW and saline environments.

Salinity acclimation had marked effects on swimming performance and respiratory metabolism in the sturgeon. The increased rates of $\dot{M}o_2$ during swimming in the fish adapted to BW relative to those in FW will have been, in part, a consequence of the increased SMR in the BW fish. However, the significantly higher net costs of locomotion in the BW sturgeon will also have contributed to the elevated $\dot{M}o_2$ during swimming in that group. The net costs of locomotion are presumably to be attributed to the costs of muscular work, and, indeed, the fish in BW exhibited significantly higher tailbeat frequencies at all speeds compared with those in FW. The increased tailbeat frequencies of the BW fish must indicate that muscular power is influenced by water salinity, presumably also as a consequence of sublethal stress.

Nonetheless, despite the metabolic load imposed by raised SMR, their increased costs of locomotion, and their reduced propulsive power, the BW animals were able to avoid a significant inhibition of U_{crit} by increasing their maximum ventilation rate, AMR, and tailbeat frequency above those of their FW siblings. That is, although the difference in AMR between the BW and FW sturgeon was not statistically significant, it could be argued that it was physiologically significant in maintaining aerobic scope and avoiding a decline in maximum performance. This adaptive strategy reveals the importance of swimming performance to the sturgeon in their natural environment. Some portion of the reduced growth rate may have been a consequence of the increased costs of locomotion in the sturgeon in BW, since captive Adriatic sturgeon exhibit constant spontaneous swimming activity at a speed of between 0.2 and 0.5 $BL \cdot s^{-1}$ (McKenzie et al. 1995).

Exercise was linked to a similar increase in plasma lactate concentration in both groups. This indicates that the increased maximum tailbeat frequency of the BW compared with the FW animals may not have been associated with increased reliance on anaerobic metabolism. Indeed, plasma lactate concentrations following exercise to exhaustion were much lower than the concentrations that are measured during exposure to graded hypoxia in this species (McKenzie et al. 1997).

Although the BW animals regulated plasma osmolality at levels similar to those of their FW counterparts, they exhibited increased plasma Na^+ and Cl^- concentrations. This may be another indication that they are in a nonoptimal osmotic environment. It is unknown what other osmolyte was regulated to maintain osmolality unchanged when the ion concentrations increased. Exercise caused a significant increase in plasma osmolality in the BW animals. This may indicate movements of water between plasma and the intracellular compartment or changes in gill permeability during maximal exercise.

In conclusion, BW at 11 $g \cdot L^{-1}$ was clearly a less optimal environment than FW for YOY Adriatic sturgeon, causing an inhibition of growth, increased metabolic rate, and increased costs of swimming. This result is consistent with previous studies indicating only partial adaptation to hyperosmotic environments by juvenile Adriatic sturgeon (Cataldi et al. 1995, 1999; McKenzie et al. 1999). All the evidence collected to date contrasts with the anecdotal reports of egg deposition in brackish estuarine waters in this species (Paccagnella 1948). Nonetheless, that larger sturgeon have been captured in the Adriatic sea (Tortonese 1989) indicates that salinity tolerance may increase with age and size, as is known to be the case for other sturgeon species (MacEnroe and Cech 1985).

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Effects of acclimation to brackish water on tolerance of salinity challenge by young-of-the-year Adriatic sturgeon (*Acipenser naccarii*)

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Abstract: The effects of a 24-h salinity challenge in water at 28 g·L^{-1} on plasma osmotic and ion homeostasis, swimming performance, and exercise respirometry were compared in young-of-the-year Adriatic sturgeon (*Acipenser naccarii*) maintained in freshwater (FW) or acclimated to brackish water (BW) that was slightly hypertonic to sturgeon plasma at a salinity of 11 g·L^{-1}. Salinity challenge caused a significant increase in plasma osmolality and Na^+ and Cl^- concentrations in both groups, but this was significantly less severe in the group acclimated to the BW as compared with FW. Salinity challenge elicited a significant and profound decline in maximum sustainable swimming speed (U_{crit}) in sturgeon from FW, whereas there was no significant effect on U_{crit} in the sturgeon acclimated to BW. A negative linear relationship was revealed between plasma osmolality, Na^+ or Cl^- concentrations, and U_{crit}. These variables appeared to influence U_{crit} by increasing costs for locomotion while reducing the sturgeon's capacity for oxygen uptake and muscular work. The results indicate that prior acclimation to mildly hypertonic BW promoted osmoregulatory adaptations that improved the ability of the sturgeon to perform exercise following further increases in water salinity.

Résumé : Les effets d'une épreuve de salinité de 24 h dans une eau à salinité de 28 g·L^{-1} sur l'homéostasie osmotique et ionique du plasma, la performance de nage et la respirométrie à l'exercice ont été comparés chez des jeunes Esturgeons de l'Adriatique (*Acipenser naccarii*) de l'année gardés en eau douce (ED) ou acclimatés à une eau saumâtre (ES) de salinité de 11 g·L^{-1}, légèrement hypertonique par rapport au plasma d'esturgeon. L'épreuve de salinité entraîne une augmentation significative de l'osmolalité du plasma et des concentrations de Na^+ et de Cl^- dans les deux groupes, mais les augmentations sont significativement plus faibles dans le groupe acclimaté à l'ES que dans le groupe d'ED. L'épreuve de salinité produit un déclin important et significatif de la vitesse maximale de nage soutenue (U_{crit}) chez les esturgeons en ED, mais reste sans effet significatif sur les poissons acclimatés à l'ES. Il existe une relation linéaire négative entre l'osmolalité du plasma, les concentrations de Na^+ ou de Cl^- et U_{crit}. Ces variables semblent influencer l'U_{crit} en augmentant les coûts de locomotion tout en diminuant l'aptitude de l'esturgeon à l'absorption d'oxygène et au travail musculaire. Nos résultats démontrent qu'une acclimatation préalable à une ES légèrement hypertonique favorise des adaptations osmorégulatrices qui améliorent l'aptitude de l'esturgeon à faire de l'exercice à des salinités encore plus élevées.

[Traduit par la Rédaction]

Introduction

The Adriatic or Italian sturgeon (*Acipenser naccarii*) has a diadromous life cycle: breeding occurs in freshwater (FW), both juveniles and adults are reported to inhabit brackish estuarine waters (Rossi et al. 1992), and adults have been captured in the Adriatic Sea (Tortonese 1989). However, the details of the Adriatic sturgeon's life history are not well understood, particularly as they are now very rare in the wild (Rossi et al. 1992). Rochard et al. (1991) classified the 37 extant species of sturgeon into three types according to life history. Type I species breed in FW and adults live and migrate for extended periods and distances in full-strength seawater (SW). Examples are the common sturgeon (*Acipenser sturio*) and Atlantic sturgeon (*Acipenser oxyrhynchus*). Type II sturgeon breed in FW but adults live in brackish water (BW) at salinities below that of SW (e.g., estuaries or the Caspian and Azov seas). Examples are the great sturgeon

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(*Huso huso*) and the Russian sturgeon (*Acipenser gueldenstaedtii*). Type III life histories are completely in FW and an example is the Siberian sturgeon (*Acipenser baerii*). Rochard et al. (1991) classified *A. naccarii* as Type II.

Recent studies on salinity tolerance in *A. naccarii* tend to support the speculation of Rochard et al. (1991). That is, although juvenile *A. naccarii* can regulate plasma ions and osmolality in hyperosmotic media, through adaptive changes such as increased activity of the Na^+,K^+-ATPase in the gills (McKenzie et al. 1999), the 96-h LC50 for salinity in 120-day-old sturgeon is approximately 23 $g \cdot L^{-1}$ (Cataldi et al. 1999), and Cataldi et al. (1995) found that juvenile Adriatic sturgeon (1–1.5 years old) could not adapt fully to SW at 30 $g \cdot L^{-1}$ for extended periods. Hypertonic BW at 11 and 20 $g \cdot L^{-1}$ inhibits growth of young-of-the-year (YOY) Adriatic sturgeon (McKenzie et al. 1999, 2001), and McKenzie et al. (2001) found that Adriatic sturgeon acclimated for 3 months to BW at 11 $g \cdot L^{-1}$, where water osmolality was only slightly hypertonic to the plasma, also exhibited elevated standard metabolic rate (SMR) and reduced swimming efficiency.

The Gulf sturgeon (*Acipenser oxyrhynchus de sotoi*) exhibits a Type I diadromous lifestyle, and life history studies on the spawning population in the Suwanee River in Florida (U.S.A.) have revealed that juveniles enter BW aged less than 1 year and remain there for 1–4 years prior to entering the sea (Sulak and Clugston 1999). This indicates that the period in BW may play a role in promoting subsequent tolerance of full-strength SW, for example by stimulating an adaptive increase in the activity of ion-translocating enzymes in the gills (McKenzie et al. 1999). Although the Adriatic sturgeon may not tolerate full-strength SW for extended periods (Cataldi et al. 1995, 1999), they are known to inhabit estuaries and have been captured in the Adriatic Sea, indicating that they must be able to tolerate SW if only for brief periods, perhaps during foraging forays into marine waters adjacent to estuaries. Thus, prior adaptation to estuarine BW may improve tolerance of such short-duration SW exposures.

In salmonids, short-duration SW transfer causes a reduction in swimming performance, which can be directly related to plasma ion levels (Houston 1959; Brauner et al. 1992). In coho salmon (*Oncorhynchus kisutch*) smolts, an improved capacity for hypoosmoregulation (when comparing wild with hatchery-reared fish) is directly linked to improved swimming performance following 24-h salinity challenge, when performance was measured as maximum sustainable swimming speed (Brauner et al. 1994). Thus, if the same were true of acipenserids, there may be a direct link between the capacity of Adriatic sturgeon to osmoregulate in hyperosmotic media and their capacity to swim and forage during brief excursions into SW.

The current study investigated the effects of short-duration (24 h) salinity challenge on plasma osmolality and ion homeostasis and the consequences for swimming performance in YOY Adriatic sturgeon. In particular, it investigated the hypothesis that prior acclimation to BW that was mildly hypertonic to sturgeon plasma, at a salinity of 11 $g \cdot L^{-1}$, would improve tolerance of a further salinity challenge and thereby ameliorate any negative effects on swimming ability. Exercise performance after salinity challenge was compared with the performance of Adriatic sturgeon as measured in their acclimation medium (i.e., FW or BW at a salinity of 11 $g \cdot L^{-1}$), as reported in a companion paper to the current study (McKenzie et al. 2001). Measurements of respiratory metabolism and tailbeat frequency were made during swimming to provide insight into the mechanisms underlying negative effects of osmotic imbalances on performance.

Materials and methods

Experimental animals

All experiments were conducted at "La Casella" Experimental Thermal Aquaculture Plant (Sarmato (PC), Italy). Two groups of 25 sibling Adriatic sturgeon were reared and stocked indoors at 23 ± 1°C in 4-m^3 fiberglass tanks (McKenzie et al. 2001). One tank received a flow of FW from a recirculating 90-m^3 biofilter and the other a flow of BW at a salinity of 11 $g \cdot L^{-1}$ from a recirculating 6-m^3 biofilter, as described in detail in McKenzie et al. (2001). Details of FW ion chemistry are provided in McKenzie et al. (2001). BW was prepared by adding marine salts (ProdacMare, Prodac, Cittadella (PD), Italy) to the FW. Feed (Trouvit Storioni, Hendrix SpA, Mozzecane (VR), Italy) was provided at 3% wet body mass·day^{-1}. Photoperiod was natural via skylights. Salinity challenge experiments were conducted on animals approximately 9 months old with a mass of approximately 200 g.

Salinity challenge

A water salinity of 28 $g \cdot L^{-1}$ was chosen for the acute 24-h salinity challenge, as suggested by Blackburn and Clarke (1987) for salmonids. This salinity represented a significant hyperosmotic challenge for the sturgeon from both FW and BW, although it is lower than that of the Adriatic Sea, which can be as high as 36 $g \cdot L^{-1}$. However, preliminary experiments revealed that acute transfer to 33 $g \cdot L^{-1}$ caused severe dehydration in animals from FW, which completely inhibited their capacity for exercise (see below). The groups of sturgeon from FW and BW that were exposed to the salinity challenge were designated as the FW/SW and BW/SW groups, respectively.

Effects of salinity challenge without exercise

Salinity challenge without exercise measured the capacity of quiescent sturgeon to maintain osmotic homeostasis. Osmotic homeostasis was measured as dehydrational weight loss, plasma osmolarity, and plasma ion concentrations. Measurements of plasma lactate were also made to gain insight into the animals' metabolic state for subsequent comparison with the measures of exercise performance. Sturgeon were rapidly dip-netted, weighed, and transferred into a 1-m^2 fiberglass tank containing approximately 250 L of water with a salinity of 28 $g \cdot L^{-1}$ in a constant-temperature chamber regulated at 23 ± 1°C. The water was prepared by adding marine salts (ProdacMare) to FW from the biofilter system at La Casella. Water in the tank was recirculated through a filter containing activated charcoal (model 1034, Eheim, Germany) to avoid any accumulation of wastes. Twenty-four hours later, the animals were anaesthetized (100 mg tricaine methanesulphonate·L^{-1}) and weighed. A 1-mL blood sample was then withdrawn from the caudal vein for measurement of plasma osmolality, Na^+, K^+, Cl^-, and lactate as described in Mckenzie et al. (2001). After sampling, fish were immediately returned to their original stocking tanks; there were no mortalities associated with the SW transfer. Six sturgeon from the BW/SW and FW/SW groups were sampled; sampling was alternated between the two groups and was completed within 45 min. The effects of salinity challenge on plasma osmolality, Na^+, K^+, and Cl^- in the FW/SW and BW/SW groups were assessed by comparison with these vari-

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ables measured in sturgeon from the FW and BW groups sampled in an identical manner but without salinity challenge, as reported in McKenzie et al. (2001).

Effects of salinity challenge on exercise performance

Salinity challenge followed by measurement of swimming performance was used to reveal the relationship between regulation of osmotic homeostasis and the capacity of the sturgeon to exercise. Individual sturgeon were rapidly dip-netted, weighed, and transferred to a modified Brett-type swimming respirometer (McKenzie et al. 2001) containing water at a salinity of 28 $g \cdot L^{-1}$. The sturgeon were acclimated to the respirometer for 22 h while swimming at a water velocity equivalent to 0.5 body length (BL)$\cdot s^{-1}$. Water velocity in the respirometer was controlled as described in McKenzie et al. (2001). Water was recirculated from the respirometer to a 200-L reservoir via a filter containing activated charcoal to avoid an accumulation of wastes from the fish. Water temperature was maintained at 23 ± 1°C by a heater with thermostat (RCS200, MGW Lauda, Lauda-Königshofen, Germany).

Following 22 h of exposure to the water at a salinity of 28 $g \cdot L^{-1}$, the sturgeon were exposed to progressive increments in swimming speed, equivalent to 0.25 BL$\cdot s^{-1}$ every 30 min, until fatigued. Fish were considered to be fatigued when they were unable to remove themselves from the posterior screen of the swimming chamber despite gentle persistent prodding. Maximum sustainable (critical) swimming speed (U_{crit}) was calculated as body lengths per second as described in McKenzie et al. (2001) using the equation provided by Brett (1964). No correction for the solid blocking effect of the fish was considered, as the total cross-sectional area of the fish did not exceed 5% of that of the swimming chamber (Bell and Terhune 1970). All swimming experiments were completed within 3 h. In order to reveal the effects of salinity challenge on U_{crit}, U_{crit} values for the FW/SW and BW/SW groups were compared with those of the FW and BW groups measured without salinity challenge, as reported in McKenzie et al. (2001).

Once fatigued, the sturgeon were gently netted out of the respirometer, rapidly weighed, and a 1-mL blood sample collected from the caudal vein to investigate the effects of salinity challenge with exercise on dehydrational weight loss and on plasma osmolarity, ion concentrations, and lactate. Measurements of oxygen uptake ($\dot{M}O_2$) were collected at each swimming speed during the exercise protocol and used to estimate SMR, active metabolic rate (AMR), aerobic scope, and net cost of locomotion, as described by McKenzie et al. (2001). Mean $\dot{M}O_2$ and net cost were also calculated at each speed for comparison between the two groups. Ventilation and tailbeat frequencies were measured at each swimming speed, as described by McKenzie et al. (2001). Ventilation frequency provided insight into the link between $\dot{M}O_2$ and ventilatory drive, while analysis of tailbeat frequency was used as an index of muscular power. Mean ventilation and tailbeat frequencies were calculated for each speed for comparison between the two groups.

In order better to understand the mechanisms underlying the effects of salinity challenge on exercise performance, selected respiratory and performance variables of the FW/SW and BW/SW groups were compared with those of the BW and FW groups measured in the absence of salinity challenge, as reported by McKenzie et al. (2001). Mean $\dot{M}O_2$, net cost, ventilation frequency, and tailbeat frequency were compared in all four groups at a common sustained speed, which was chosen to be 1.5 BL$\cdot s^{-1}$. Also, SMR, AMR, aerobic scope, maximum ventilation frequency, and maximum tailbeat frequency were compared among the four groups.

Statistical analysis

All statistical analyses were performed with the Sigmastat software package (Jandel Scientific, San Rafael, Calif.). The effect of salinity challenge on wet mass was assessed within a group by paired t test. The effects of salinity challenge on plasma variables were assessed by comparing the four groups (FW, FW/SW, BW, and BW/SW) with a two-way analysis of variance (ANOVA) where water salinity was the primary factor and exercise to fatigue an interacting factor. Bonferroni post hoc t tests were used to identify significant differences between means. To describe the relationships between swimming speed and respirometric or performance variables during the exercise protocol, linear, exponential, or power functions were applied and the function with the highest correlation coefficient identified. Comparison of performance or metabolic variables among the four groups was by one-way ANOVA with Bonferroni post hoc t tests. In all cases, $P < 0.05$ was taken as the fiducial level of significance.

Results

Table 1 presents the mass and length of the sturgeon from the FW, FW/SW, BW, and BW/SW groups. The data for the FW and BW groups are as reported by McKenzie et al. (2001). There were no significant differences in mass or length of the FW/SW compared with the BW/SW group. However, the length of the FW and BW animals in which McKenzie et al. (2001) studied exercise performance was slightly but significantly less than the length of the FW/SW and BW/SW groups that were studied for the effects of salinity challenge on exercise performance in the current study. This was a consequence of the rapid growth rates of the sturgeon during completion of the two studies (McKenzie et al. 2001).

Effects of salinity challenge on body mass, plasma osmotic homeostasis, and lactate concentration

Salinity challenge without exercise caused a significant weight loss in both the FW/SW and BW/SW groups, but the loss was significantly greater in FW/SW fish (Table 2). The effects of salinity challenge on plasma osmolality, Na^+, Cl^-, and K^+ are presented in Fig. 1. With regard to the effects of salinity challenge on animals that were not exercised to fatigue, there was a significant increase in osmolality, Na^+, Cl^-, and K^+ in both the FW/SW and BW/SW groups relative to the FW and BW groups, respectively. However, following the challenge, the BW/SW animals showed significantly lower levels for all four variables than did the FW/SW sturgeon.

Exercise to fatigue caused no significant changes in the plasma variables of any group except the BW/SW group, where it caused an increase in plasma Na^+ and Cl^- relative to the same group without exercise (Fig. 1). However, in the FW/SW group, exercise to fatigue was linked to a further significant, and profound, weight loss (Table 2). Exercise did not exacerbate significantly the effects of salinity challenge on weight loss in the BW/SW group (Table 2).

Salinity challenge without exercise elicited a significant increase in plasma lactate in the FW/SW sturgeon that was not, however, observed in the BW/SW group, although the mean lactate concentrations were not significantly different between the two groups following the salinity challenge (Table 2). Salinity challenge plus exercise to fatigue had no further effect on plasma lactate in the FW/SW group but caused

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Table 1. Mean (±SE) mass and length of the Adriatic sturgeon from the four experimental groups as measured immediately prior to their use in any experiment.

Group	*N*	Mass	Length
FW	6	167±15 *a*	28.6±0.3 *ab*
FW, exercise	8	156±12 *a*	26.3±0.4 *a*
BW	6	163±17 *a*	27.8±0.4 *ab*
BW, exercise	9	158±16 *a*	26.9±1.0 *a*
FW/SW	6	197±14 *a*	29.8±0.5 *b*
FW/SW, exercise	6	208±14 *a*	30.7±0.6 *b*
BW/SW	6	204±15 *a*	29.9±0.5 *b*
BW/SW, exercise	6	198±15 *a*	29.7±0.4 *b*

Note: FW/SW is the FW group exposed to a 24-h salinity challenge and BW/SW is the BW group exposed to a salinity challenge. "Exercise" implies that the fish were to be used in the measurements of exercise performance. A common letter indicates no significant difference (one-way ANOVA, $P > 0.05$). Data for FW and BW groups are as reported by McKenzie et al. (2001).

Table 2. Effects of a 24-h salinity challenge at 28 g·L^{-1}, with or without exercise to fatigue, on mean (±SE) weight loss and plasma lactate in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1}.

	Weight loss (%)	Plasma lactate (mmol·L^{-1})
FW, no exercise		0.58±0.05
FW/SW, no exercise	14.4±2.0*#	1.20±0.09‡
FW/SW, at fatigue	23.1±2.8*#†	1.52±0.11
BW, no exercise		0.70±0.03
BW/SW, no exercise	6.7±1.1*	1.21±0.10
BW/SW, at fatigue	11.1±0.8*	1.81±0.23†

Note: FW/SW is the FW group exposed to a salinity challenge and BW/SW is the BW group exposed to a salinity challenge. $N = 6$ in all cases. *Significant weight loss (paired *t* test); #significantly different from the BW/SW group (one-way ANOVA); †significantly different from the same group without exercise (one-way ANOVA); ‡significantly different from the FW group (one-way ANOVA). $P < 0.05$ in all cases. Data for the FW and BW groups are as reported by McKenzie et al. (2001).

a further increase in lactate concentration in the BW/SW group (Table 2).

Effects of salinity challenge on exercise performance

Following salinity challenge, the FW/SW group exhibited a significantly lower U_{crit} (P = 0.004) than the BW/SW group when the two groups were compared by *t* test (Fig. 2). When all four groups (FW, FW/SW, BW, and BW/SW) were compared by ANOVA, the FW/SW group had a significantly lower U_{crit} than the FW group, but there were no differences between the BW and BW/SW groups. However, the BW/SW group had a significantly lower U_{crit} than the FW group, and the FW/SW group had a significantly lower U_{crit} than the BW group (Fig. 2).

A comparison of mean U_{crit} with the mean values of plasma osmolality, Na^+, and Cl^- in the four groups at fatigue revealed clear negative linear relationships with correlation coefficients above 0.99 ($R^2 > 0.98$) in all three cases (Fig. 3). There were also significant linear relationships between individual values for U_{crit} and for plasma osmolality, Na^+, or Cl^- in the FW, BW, FW/SW, and BW/SW groups following exercise. The correlation coefficients for these relationships were much lower than when comparing the mean values. The highest coefficient, with an R^2 of 0.548 ($P < 0.0001$), was observed for Na^+, where $U_{crit} = -0.023[Na^+] + 6.4$, where U_{crit} is in body lengths per second and $[Na^+]$ in milliequivalents per litre.

Following salinity challenge, the relationship between swimming speed and $\dot{M}O_2$ was quite variable in fish from both the FW/SW and BW/SW groups, with the relationships described best by exponential equations (Table 3). There were no differences between these two groups in their mean $\dot{M}O_2$ at any speed. The FW/SW and BW/SW groups also had similar SMR, AMR, and aerobic scope (Table 4). The relationship between swimming speed and net cost of locomotion was described by a power function in both groups (Table 3), and although mean net costs at all speeds were higher in the FW/SW group, the differences were not statistically significant. Both groups exhibited an increase in ventilation with increased swimming speed, but the FW/SW group exhibited significantly higher ventilation rates than the BW/SW group at 0.75, 1.0, and 1.25 BL·s^{-1} (Fig. 4). Both the FW/SW and BW/SW groups had similar, linear relationships between tailbeat frequency and swimming speed with no difference between the groups (Table 3).

The comparison of respiratory and performance variables between the FW, FW/SW, BW, and BW/SW groups, when swimming at a common sustained speed of 1.5 BL·s^{-1}, revealed some significant differences (Fig. 5). Thus, the FW/SW, BW, and BW/SW groups exhibited significantly higher mean $\dot{M}O_2$ than that measured in the FW group (Fig. 5). Net costs of locomotion were also significantly higher in these same groups as compared with the FW group (Fig. 5). The FW/SW, BW, and BW/SW groups all had significantly higher ventilation rates than the FW group (Fig. 5). Tailbeat frequency was significantly higher in the BW group than in the FW, FW/SW, and BW/SW groups (Fig. 5).

There were also differences between the four groups in other measures of metabolism and performance. The AMR and aerobic scope of the FW/SW and BW/SW groups were significantly lower than those of the BW group (Table 4). Maximum ventilation rate was significantly higher in the FW/SW and BW groups than in the FW group (Table 4). Maximum tailbeat frequency was significantly lower in the two salinity challenge groups as compared with both the BW and FW groups, while it was significantly higher in the BW group compared with the FW group (Table 4).

Discussion

The data indicate that prior acclimation to BW at a salinity of 11 g·L^{-1} improved tolerance of hyperosmotic salinity challenge in YOY Adriatic sturgeon. This improved tolerance was revealed as less severe dehydration (measured as decline in body mass) in the BW/SW group compared with the FW/SW group following 24 h of exposure to water at 28 g·L^{-1}, as a consequence of the better homeostatic regulation of plasma osmolality and ion levels in the BW/SW group. The improved homeostatic osmoregulation in the BW/SW versus FW/SW group following salinity challenge was presumably a consequence of adaptive responses during prior acclimation to mildly hypertonic BW. A linear increase

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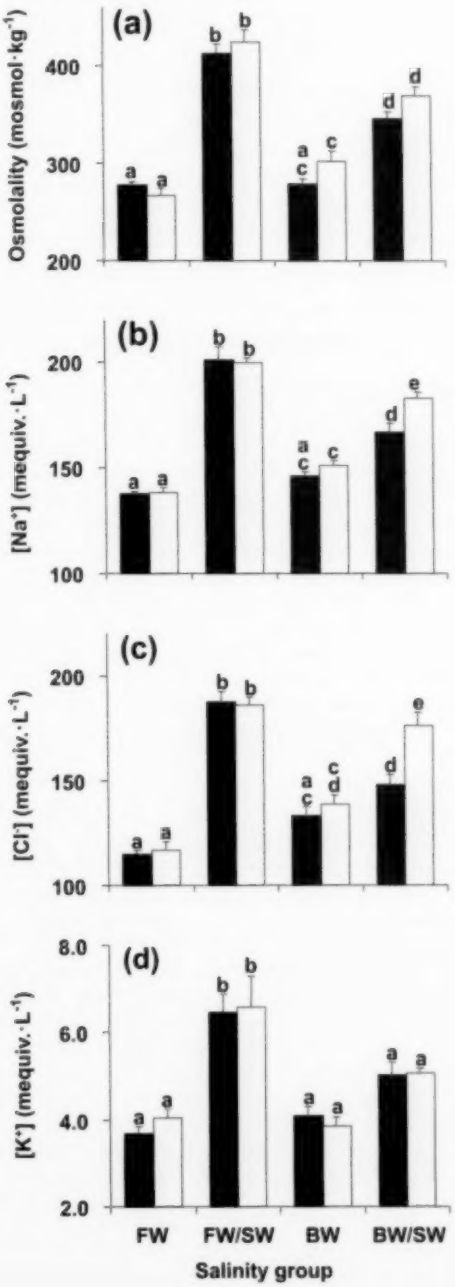
Fig. 1. Mean (±SE) (*a*) plasma osmolality and (*b*) Na^+, (*c*) Cl^-, and (*d*) K^+ concentrations in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1} and in sturgeon from these two groups following a 24-h salinity challenge at 28 g·L^{-1} (FW/SW and BW/SW, respectively). Data are presented both for resting animals (solid bars) and for animals that were exercised to fatigue (open bars). $N = 6$ in all cases except FW following exercise where $N = 8$ and BW following exercise where $N = 9$. A common letter indicates no significant difference between groups (two-way ANOVA, $P > 0.05$). Data for FW and BW are replotted from McKenzie et al. (2001).



in gill Na^+,K^+-ATPase activity with increasing acclimation salinity has previously been described in YOY Adriatic sturgeon, although the increased activity was not statistically significant in fish acclimated to BW at 11 g·L^{-1} when compared with those in FW (McKenzie et al. 1999).

The effects of prior acclimation to BW on subsequent tolerance of a 24-h salinity challenge in the sturgeon contrast with results in juvenile salmonids, where such prior BW acclimation had no effect on regulation of plasma fluid and ion homeostasis upon SW challenge (Morgan and Iwama 1991). The results indicate, however, that the period that the juveniles of diadromous acipenserids spend in BW prior to moving into the sea (Sulak and Clugston 1999) may be important in promoting adaptive mechanisms for hypoosmoregulation.

The improved regulation of plasma osmotic status was accompanied by improved exercise performance, revealed as a higher U_{crit} in the BW/SW as compared with the FW/SW group and by the fact that the BW/SW group had performance similar to that of the BW group, while the FW/SW group exhibited a profound decline in U_{crit} when compared with the FW group. Some proportion of the decline in U_{crit} between the sturgeon exposed to salinity challenge and their siblings swum in their acclimation media may have been attributable to increased body length in the FW/SW and BW/SW groups. The U_{crit} in fish should be proportional to length raised to the power of 0.5, based on the assumption that fish volume and muscle mass increase with the square of length (Beamish 1978). This effect of length on U_{crit} has been proven experimentally for salmonids (Brett 1965). Application of the same relationship to the sturgeon in the current study revealed that increased length accounted for 10% of the decline in U_{crit} in the FW/SW group relative to the FW group and for 16% of the reduction in the BW/SW versus BW group. Correction for these effects of length did not influence the outcome of the statistical comparison among the four groups. Therefore, the significant differences in U_{crit} can be attributed to the effects of the salinity challenge and associated osmotic imbalances.

Correction for small effects of length also had no effect on the linear relationships between mean U_{crit} and mean plasma osmolality, Na^+, or Cl^- in the four groups of sturgeon; the correlation coefficients for the corrected relationships remained extremely high ($R^2 = 0.97$ in all three cases). These direct relationships are a clear indication that regulation of plasma osmotic status during changes in environmental salinity is of direct functional consequence for this species. Somewhat similar relationships between mean plasma ion concentrations and mean U_{crit} following changes in environmental salinity have been described in salmonids (Brauner et al. 1992, 1994). Brauner et al. (1992) found that an increase in plasma Na^+ following salinity challenge was linked to reduced U_{crit} in coho salmon parr, but they found that the overall relationship was described by a bell-shaped second-order polynomial, with a clear maximum for U_{crit} at an optimum plasma Na^+ of 145 mequiv.·L^{-1}, the plasma Na^+ level found in FW, and a significant decline in U_{crit} at a plasma Na^+ of above 175 mequiv.·L^{-1}. In the current study, a linear relationship was found across the concentrations of Na^+ measured, such that any increase in plasma Na^+ above that measured in sturgeon acclimated to FW was associated with some decline in U_{crit}. The decline relative to the FW

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Fig. 2. Mean (±SE) U_{crit} in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1} and in sturgeon from these two groups following a 24-h salinity challenge at 28 g·L^{-1} (FW/SW and BW/SW, respectively). N = 6 for FW/SW and BW/SW, N = 8 for FW, and N = 9 for BW. A common letter indicates no significant difference between groups (one-way ANOVA, $P > 0.05$). Data for FW and BW are replotted from McKenzie et al. (2001).

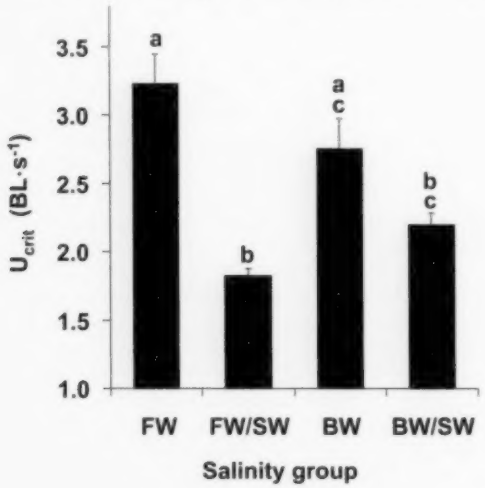
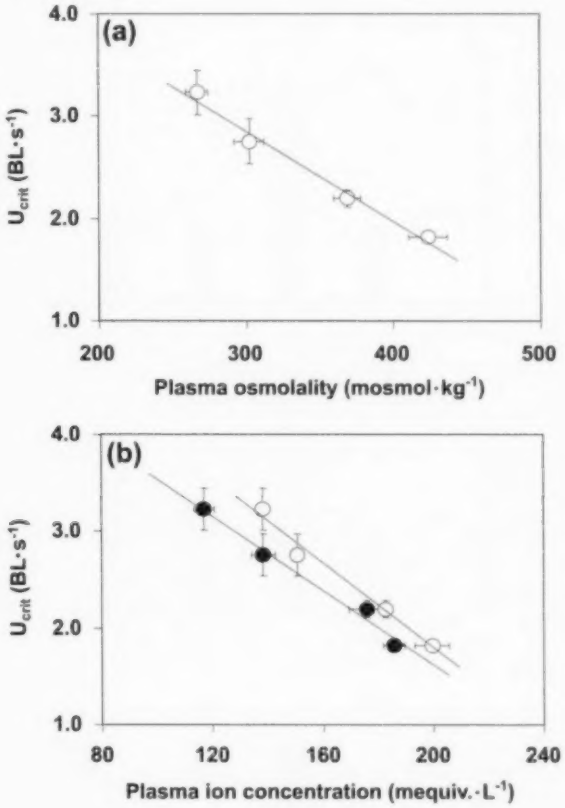


Fig. 3. Least-squares linear regression analysis describing the relationship of mean (±SE) critical swimming speed (U_{crit}) versus mean (±SE) (*a*) plasma osmolality and (*b*) Na^+ (open circles) and Cl^- (solid circles) measured in the Adriatic sturgeon following exercise to fatigue. For both panels, when reading from left to right down the regression lines, the circles represent the FW group, BW group, BW group following salinity challenge, and FW group following salinity challenge, respectively (see text for further details).



group was significant in the BW/SW group, where mean plasma Na^+ was 169 mequiv.·L^{-1} in nonexercised animals and 188 mequiv.·L^{-1} in fish exercised to fatigue. Thus, these results are consistent with those found for salmonids (Brauner et al. 1992) in terms of the effects of increasing plasma Na^+ on swimming performance. It is likely that, as described by Brauner et al. (1992) for coho salmon, there is also an optimum Na^+ for sturgeon, with U_{crit} declining if plasma Na^+ falls below the levels measured in FW (e.g., if the sturgeon were placed in distilled water).

When considering the data from individual sturgeon, that plasma Na^+ should have shown a closer relationship to U_{crit} than osmolality or Cl^- is also consistent with the data reported by Brauner et al. (1992) for coho salmon and indicates that regulation of extracellular Na^+ levels is of primary importance in determining exercise performance following salinity challenge. Nonetheless, although the relationship was significantly linear, the level of plasma Na^+ accounted for only about 55% of the variability in U_{crit}, indicating that the regression analysis provided only limited predictive power for individual sturgeon.

The mechanisms underlying the negative effects of osmotic imbalances on swimming ability are not known, but as U_{crit} is dependent on aerobic muscular work (Brett 1964), some insights can be gained by analysis of the characteristics of Mo_2, ventilation, and tailbeat frequency during exercise. The FW/SW, BW, and BW/SW groups all showed increased Mo_2 and net cost of locomotion relative to the FW group when compared at a sustained speed of 1.5 BL·s^{-1}, and these were linked to significantly higher rates of ventilation in those groups. This indicates a reduced swimming efficiency in the FW/SW, BW, and BW/SW groups and can therefore explain their poorer performance relative to the FW group. In the BW group, the increased costs of locomotion could be accounted for by their increased tailbeat frequencies relative to the other groups (McKenzie et al. 2001). This negative effect on propulsive power of long-term acclimation to BW is difficult to explain in terms of any osmotic variable and may have been a consequence of an underlying stress response (McKenzie et al. 2001). The increased costs of locomotion in the FW/SW and BW/SW groups were not associated with increased tailbeat frequencies, and their reduced swimming efficiency may have been a consequence of a direct effect of increased ion concentrations on muscle contractility (Homsher et al. 1974) and on the neuromuscular apparatus (Houston 1959; Brauner et al. 1992).

As described by McKenzie et al. (2001), the BW fish were able partially to compensate for the effect that their increased swimming costs might have had on U_{crit} by increasing maximum ventilation frequency and maximum Mo_2, thereby preserving aerobic scope and achieving a higher maximum tailbeat frequency (McKenzie et al. 2001). The FW/SW and BW/SW groups were unable to make such compensatory ad-

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Table 3. Equations describing the exponential relationship between swimming speed and M_{O_2}, the power relationship between swimming speed and net cost of locomotion (as M_{O_2}), and the linear relationship between tailbeat frequency and swimming speed in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1} and then exposed to a 24-h salinity challenge at 28 g·L^{-1} (FW/SW and BW/SW, respectively).

	Group	Equation	Number of observations	R^2
Swimming speed vs. M_{O_2}	FW/SW	$y = 227e^{0.450x}$	35 on 6 fish	0.415
	BW/SW	$y = 254e^{0.301x}$	44 on 6 fish	0.482
Swimming speed vs. cost of locomotion	FW/SW	$y = 112.1x^{1.135}$	35 on 6 fish	0.622
	BW/SW	$y = 93.1x^{1.039}$	44 on 6 fish	0.601
Tailbeat frequency vs. swimming speed	FW/SW	$y = 0.856x - 0.71$	36 on 6 fish	0.783
	BW/SW	$y = 0.847x - 0.571$	47 on 6 fish	0.762

Note: Swimming speed in BL·s^{-1} M_{O_2}, in mg·kg^{-1}·h^{-1}, and tailbeat frequency in Hz.

Table 4. Mean (±SE) values for exercise-related aspects of respiratory metabolism and performance in Adriatic sturgeon acclimated to FW or BW at 11 g·L^{-1} or in these groups when subjected to a 24-h salinity challenge at 28 g·L^{-1} (FW/SW and BW/SW, respectively).

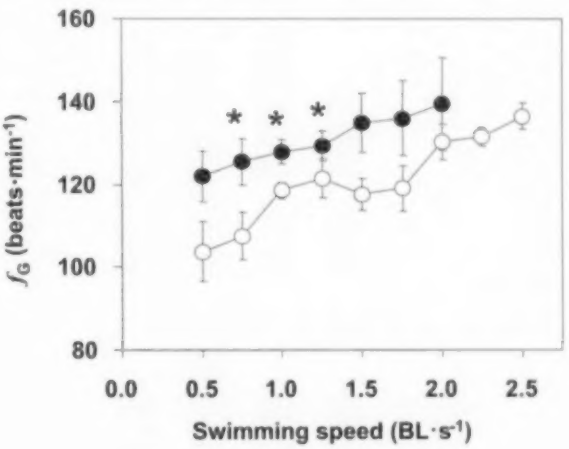
	FW	FW/SW	BW	BW/SW
SMR (mg O_2·kg^{-1}·h^{-1})	216±25	243±42	281±11	251±36
AMR (mg O_2·kg^{-1}·h^{-1})	548±61	502±35*	635±22	476±15*
Aerobic scope (mg O_2·kg^{-1}·h^{-1})	332±47	239±45*	354±20	225±30*
Maximum tailbeats (Hz)	3.91±0.15*	2.95±0.13*#	4.48±0.15	2.77±0.14*#
Maximum ventilation (beats·min^{-1})	101±2.1	135±7.1 #	123±3.7 #	118±4.8

Note: $N = 8$ for FW, $N = 9$ for BW, and $N = 6$ for FW/SW and BW/SW. *Significantly different from the BW group (one-way ANOVA); #significantly different from the FW group (one-way ANOVA). $P < 0.05$ in all cases. Data for the FW and BW groups are as reported by McKenzie et al. (2001).

justments for their increased swimming costs and exhibited reduced maximum M_{O_2} and aerobic scope relative to the BW group and reduced maximum tailbeat frequencies relative to both the FW and BW groups, despite elevated maximum ventilation rates. This is indicative of a reduced capacity to deliver oxygen to the working muscles. The sturgeon exposed to salinity challenge were severely dehydrated, and dehydration of the gills following SW exposure (Stagg et al. 1989; Eddy and Chang 1992) could compromise M_{O_2} (Brauner et al. 1992). Oxygen delivery to the tissues may also have been compromised because of direct effects of hypernatremia on cardiac function, as has been demonstrated in toads (Hillman and Withers 1988). Short-duration SW exposure causes a reduction in oxygen content of the blood in salmonids (Maxime et al. 1991), possibly as a consequence of the effects of plasma Na^+ and osmolality on haematocrit (Brauner et al. 1992) and the effects of increased red cell Cl^- concentrations on haemoglobin–oxygen affinity (Maxime et al. 1991).

However, the analysis of exercise-related metabolism in the sturgeon following salinity challenge did not reveal any clear mechanisms for the difference in performance between the FW/SW and BW/SW groups. That is, during exercise, the FW/SW fish had the highest plasma osmolality and ion concentrations, which must ultimately have been the reason for their poorer performance relative to the BW/SW group, given the relationship between these plasma variables and U_{crit}. However, this was not linked to any significant differences between these groups in their SMR, AMR, aerobic scope, costs of locomotion, or tailbeat frequencies. This contrasts with the results obtained for the FW and BW groups when exercised in their acclimation media (McKenzie et al. 2001), where there were significant differences in SMR, costs of locomotion, and tailbeat frequencies that were not, however, associated with any significant difference in U_{crit}.

Fig. 4. Relationship between mean (±SE) gill ventilation rate (f_G) and swimming speed in Adriatic sturgeon acclimated to FW or BW and then exposed to a 24-h salinity challenge at 28 g·L^{-1} (FW/SW (solid circles) and BW/SW (open circles), respectively). For the FW/SW group, $N = 6$ at all speeds except 2.0 BL·s^{-1} where $N = 2$. For the BW/SW group, $N = 6$ at all speeds except 2.0 BL·s^{-1} where $N = 4$ and 2.25 BL·s^{-1} where $N = 2$. An asterisk indicates significantly higher than the BW group at the same swimming speed (t test, $P < 0.05$).



This may be because the animals in the FW/SW and BW/SW groups had not achieved a steady-state homeostatic condition at 24 h following the acute salinity challenge but were subject to a number of effects occurring simultaneously, with the potential of masking interactions between effects. The FW/SW group did exhibit higher ventilation rates than the BW/SW group, which is indicative of a re-

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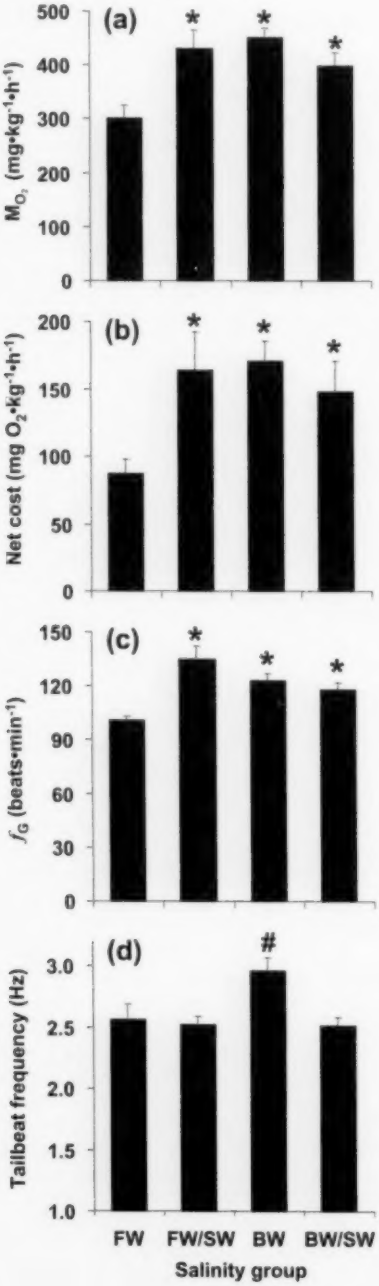
Fig. 5. Mean (±SE) (*a*) Mo_2, (*b*) net cost of locomotion (as Mo_2), (*c*) gill ventilation rate (f_G), and (*d*) tailbeat frequency in Adriatic sturgeon swimming at a sustained speed of 1.5 BL·s^{-1}. Data are presented for sturgeon acclimated to FW or BW and for sturgeon from these two groups following a 24-h salinity challenge at 28 g·L^{-1} (FW/SW and BW/SW, respectively). $N = 6$ for FW/SW and BW/SW, $N = 8$ for FW, and $N = 9$ for BW. An asterisk indicates significantly different from the FW group and a pound sign indicates significantly different from all other groups (one-way ANOVA, $P < 0.05$). Data for FW and BW are replotted from McKenzie et al. (2001).



duced capacity to meet tissue oxygen demands, and also exhibited a further profound dehydrational weight loss during exercise.

In nature, diadromous sturgeon are reported to spend periods of their juvenile life in BW prior to moving offshore to forage in the marine environment (Sulak and Clugston 1999) for periods that may be species dependent (Rochard et al. 1991). For the Adriatic sturgeon, although adults have been captured in the Adriatic Sea (Rossi et al. 1991), there is evidence that they are not able to tolerate full-strength SW for extended periods (Cataldi et al. 1995, 1999; McKenzie et al. 1999) such that periods in the sea may only be brief and linked to foraging activities. If these occur in the FW cone of a river, the sturgeon can be expected to encounter sudden differences in water salinity. The results of the companion study (McKenzie et al. 2001) indicate that acclimation to slightly hypertonic BW carries a metabolic cost for YOY Adriatic sturgeon that is associated with slightly reduced growth rates. However, the results of the current study indicate that such acclimation to BW improves the capacity of the sturgeon to regulate plasma osmolality and ion concentrations following SW exposure and would thereby protect their capacity to exercise and forage when they encounter short-term increases in water salinity.

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Prey size of salmonid fishes in streams, lakes, and oceans

Ernest R. Keeley and James W.A. Grant

Abstract: The growth rate of salmonid fishes is typically fastest in oceans, intermediate in lakes, and slowest in streams. We compiled literature data to test whether differences in the size of prey eaten by salmonids in the three habitats could account for these differences in growth rate (i.e., the prey-size hypothesis). In all three habitats, salmonid fishes exhibited ontogenetic niche shifts from feeding primarily on invertebrates when small to feeding on fishes when large. Contrary to the prey-size hypothesis, invertebrates eaten in streams were larger than those eaten in lakes or oceans, whereas fish eaten in oceans were smaller than those eaten in streams or lakes. Consistent with the prey-size hypothesis, salmonids began eating fish at a smaller size in oceans (8 cm) than in lakes (15 cm) or streams (27 cm). However, the size at which salmonids became predominantly piscivorous (31 cm) did not differ significantly between habitats. We suggest that the difference in growth potential between the three habitats is partly related to the size at which fish first enter the diet.

Résumé : Le taux de croissance des poissons salmonidés est typiquement le plus rapide dans les océans, intermédiaire dans les lacs et le plus faible dans les cours d'eau. Une compilation de données de la littérature a permis de vérifier si les différences dans les tailles des proies mangées par les salmonidés dans ces trois habitats peuvent expliquer les variations dans les taux de croissance (i.e. l'hypothèse de la taille des proies). Dans les trois habitats, les salmonidés ont opéré un glissement ontologique de niche, se nourrissant d'invertébrés lorsqu'ils sont petits et, une fois grands, de poissons. Contrairement à ce que prédit l'hypothèse de la taille des proies, les invertébrés consommés dans les cours d'eau sont plus grands que ceux mangés dans les lacs ou les océans, alors que les poissons consommés dans les océans sont plus petits que ceux pris dans les lacs et les cours d'eau. En accord avec l'hypothèse, cependant, les salmonidés commencent à manger des poissons à une taille plus petite dans les océans (8 cm) que dans les lacs (15 cm) ou les cours d'eau (27 cm). Néanmoins, la taille à laquelle les salmonidés deviennent en prédominance des piscivores (31 cm) ne diffère pas significativement d'un habitat à l'autre. Nous posons l'hypothèse que la différence du potentiel de croissance entre les trois habitats est reliée à la taille à laquelle les poissons commencent à faire partie du régime alimentaire.

[Traduit par la Rédaction]

Introduction

Salmonid fishes live in a variety of habitats that can be conveniently classified as streams, lakes, and oceans (Scott and Crossman 1973). While all individuals spawn in fresh water, there is considerable variation in how much time is spent in their natal habitat (Hoar 1976; Randall et al. 1987). At one extreme, some populations complete their life cycle within a stream or lake, while others routinely migrate between two or three of the habitat types (Scott and Crossman 1973; Hoar 1976).

Evolutionary explanations for these often spectacular migrations usually focus on strategies to increase growth rate and, ultimately, reproductive success (Gross 1987). Salmonid fishes typically grow faster in lakes than in streams (Bachman 1982; Hutchings 1986; but see Gulseth and Nilssen 1999) and oceans than in fresh water (Gross 1987; Økland et al. 1993). Two hypotheses, which are not mutually exclusive, have been proposed to explain why salmonids grow faster in some habitats than in others: (1) differences in overall productivity or (2) differences in mean prey size available. For example, the migration of salmonids to oceans and the resulting increase in growth rate is thought to be related to the greater productivity of marine versus freshwater ecosystems in north-temperate latitudes (Gross et al. 1988). The prey-size hypothesis seems to originate from studies of salmonids in fresh water, in which details of their feeding biology are well known. Because of the energetic advantage of feeding on larger prey (e.g., Kerr 1971; Wańkowski and Thorpe 1979), salmonids grow larger when large prey are available (Martin 1970; Trippel and Beamish 1993; Mittelbach and Persson 1998). Moreover, the slow growth of salmonids that often occurs in streams (Bachman 1982) and sometimes in lakes (Konkle and Sprules 1986) is thought to occur because of a lack of suitable prey sufficiently large to sustain further growth (Kerr 1971). To increase the size of salmonids for trophy fishing, large prey, such as *Mysis* spp. or forage fish, were often introduced into lakes, even though such introductions typically

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decreased the overall yield of the target population (Northcote 1991; Rasmussen et al. 1990).

Despite hundreds of individual studies of the diet of salmonid fishes, no general synthesis of their feeding biology in different habitats is available. We decided to focus on two variables that are often quantified and, hence, comparable in many studies: the taxonomic composition of the diet and the mean prey size eaten. In addition, we use the latter to test the prey-size hypothesis. If prey size is the primary factor responsible for variation in the growth rate in salmonid fishes, then prey size should follow the trends in growth potential of the three habitat types (i.e., oceans > lakes > streams). In particular, salmonid fishes may switch to eating fish at a smaller size in oceans than in lakes or streams. Because ontogenetic shifts in diet are almost universal in fishes (Werner and Gilliam 1984), including salmonids (Keeley and Grant 1997; Mittelbach and Persson 1998), we take an allometric approach when comparing the prey size in the three habitats. We also compare the allometric trends in salmonid prey size with morphological constraints, such as gill-raker spacing and mouth size, which may limit the minimum size of prey eaten (Wańkowski 1979; Damsgård 1995), and with general allometric relationships for animals (Cohen et al. 1993).

Materials and methods

Literature search and data collection

We searched the literature for studies reporting quantitative descriptions of the diet of salmonid fishes that are commonly referred to as charr, salmon, and trout. We began by searching volumes 1–54 of the Canadian Journal of Fisheries and Aquatic Sciences (formerly the Journal of the Fisheries Research Board of Canada), volumes 63–96 of the Fishery Bulletin of the U.S. National Marine Fisheries Service, volumes 1–17 of the North American Journal of Fisheries Management, and volumes 51–126 of the Transactions of the American Fisheries Society. We compiled all articles that mentioned some aspect of the diet of a salmonid fish and examined all other available articles on diet that were cited by the articles in our original search.

Because we hypothesized that body size would be an important determinant of prey size, we collected data only from studies that provided a paired measurement of the body size of a salmonid fish and its prey. We used predator and prey lengths as our measures of size in all analyses, because these were the most commonly available data. Most studies reported the length of a fish as fork length rather than as total length. In the few studies where authors did not specify which length measurement was used, we assumed they were using fork length. In cases where the mass of a fish was given but not the length, we used species-specific length–mass regressions from Carlander (1969) to convert mass into length. We did not use studies that reported prey size in mass, because of the great variability in length–mass regressions between groups of invertebrates. Only studies that reported size estimates of the majority of prey items in the diet of a fish were included.

In recording data from published studies, we used the average lengths of the predator and prey, when authors reported these values in summary statistics, or interpolated numerical values from figures. If a range of predator and prey sizes were reported but not means, we used the midpoint in our analyses. In cases where two or more size- or age-classes of fish were reported, we included a datum for each class of fish. Similarly, if more than one species was examined by a particular study, we recorded a datum for each species. Our method of extracting data allowed us to include more than one datum per study but guarded against over-representing intensive studies in the data set. Because our method ignores inter-individual variability in diet within populations (e.g., see McLaughlin et al. 1994), our predictive relationships represent differences between the typical individuals in different populations.

Diet composition was also compiled from studies providing quantitative measures of predator and prey lengths. We used the average number of different types of prey in fish stomachs to quantify diet composition, because it was the most common method in the literature. Because studies varied widely in the precision of the taxonomic identification of stomach contents, we categorized prey as fish, crustaceans, insects, and other (mollusks, plants, amphibians, mammals, and detritus). For each size category of fish in a particular study, we recorded the average percentage of the stomach contents comprising each of these four categories. Habitat was included as a categorical variable in the analyses: streams—any freshwater lotic habitat; lakes—any freshwater lentic habitat; and oceans—any marine habitat, including estuaries.

Statistical analyses

The relationship between predator size and prey size was investigated using an ordinary least-squares (OLS) regression for predictive relationships and reduced major axis (RMA) regression for descriptive analyses (McArdle 1988). We used the standard error of the OLS regression to approximate the standard error of the RMA regression as recommended by Sokal and Rohlf (1995). Because body-size distributions tend to be lognormally distributed, we $\log_{10}$-transformed body size variables to meet the assumption of homogeneity of variance. We compared the predator-length versus prey-length data with two morphological constraints that may limit the size range of prey for salmonid fishes. Damsgård's (1995) allometric equation for maximum prey length for brown trout (*Salmo trutta*; prey length (cm) = 0.535 predator length (cm) – 0.487) was used to predict the upper limit of prey size, whereas Wańkowski's (1979) allometric equation of gill-raker spacing for Atlantic salmon (*Salmo salar*; gill-raker spacing (mm) = 0.115 fork length (cm)) was used to predict the lower limit of prey size. We used logistic regression to predict the shift from an invertebrate to a fish diet that often happens as fish grow (Mittelbach and Persson 1998). We defined fish as piscivores in two ways: if there were any fish in the diet or if fish accounted for ≥60% of the diet.

The data presented in the figures represent untransformed values. For some comparisons, we replotted arithmetic relationships from the literature on a logarithmic scale. We used $\alpha = 0.05$ as a critical level of significance. Residuals of final regression models were examined for consistency of error variance across the full range of all independent variables.

Results

We used data from 93 different studies, representing 19 species or subspecies of salmonid fishes (Table 1). Of these 93 studies, 39, 32, and 23 described fish from streams, lakes, and oceans, respectively. There were six different geographic regions represented in the data set, 76.1% of the observations came North America, 16.2% from Europe, 2.7% from New Zealand, 0.6% from Australia, 2.7% from Japan, and 1.2% from Scandinavia. Any single study contributed an average of 1.1% of the observations to the data set, and never contributed more than 13.6% of the total number of observations in the data set.

Prey composition

Eighty-one studies provided information on the taxonomy of organisms in the diet of salmonid fishes, based on the

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Table 1. Scientific and common names of salmonid fishes used in analyses of prey composition and prey size, and reference sources for data.

Scientific name	Common name	Source(s)[a] for composition	Source(s)[a] for size
Hucho hucho	Danube salmon	63, 90	63, 80, 90
Oncorhynchus clarki	Cutthroat trout	65	73, 89
Oncorhynchus clarki clarki	Coastal cutthroat trout	69	69
Oncorhynchus clarki pleuriticus	Colorado River cutthroat trout	9	9
Oncorhynchus gorbuscha	Pink salmon	6, 31, 34, 44, 47, 62	6, 31, 35, 47, 62
Oncorhynchus keta	Chum salmon	6, 26, 31, 44, 53, 62	6, 26, 31, 35, 41, 62
Oncorhynchus kisutch	Coho salmon	6, 10, 11, 25, 43, 61, 62, 91	11, 43, 61, 62, 70
Oncorhynchus masou ishikawai	Red-spotted masu salmon		64
Oncorhynchus mykiss	Rainbow trout	5, 8, 12, 20, 36, 38, 42, 57, 65, 68, 71, 76, 79, 83, 85, 92	2, 5, 8, 32, 42, 55, 57, 68, 76, 79
Oncorhynchus nerka	Sockeye salmon	4, 11, 74, 79	21, 35, 79
Oncorhynchus tshawytscha	Chinook salmon	5, 10, 13, 25, 27, 46, 57, 72, 75, 78	11, 17, 27, 56, 75, 78
Salmo salar	Atlantic salmon	18, 22, 45, 60, 84	3, 18, 22, 45, 60, 84
Salmo trutta	Brown trout	7, 14, 23, 28, 30, 32, 33, 37, 40, 48, 49, 66, 77	2, 7, 14, 30, 32, 37, 33, 48, 49, 55, 77
Salvelinus alpinus	Arctic charr	15, 51, 59	59
Salvelinus confluentus	Bull trout		81
Salvelinus fontinalis	Brook trout	1, 4, 50, 54, 76, 84, 92	4, 54, 76, 84
Salvelinus leucomaenis	Japanese charr	29	
Salvelinus malma	Dolly Varden	65, 91	81
Salvelinus namaycush	Lake trout	16, 19, 39, 52, 58, 67, 82, 86, 87, 88	16, 24, 52, 58, 86, 93

[a]Sources of data are defined by numbers as follows: 1, Allan (1981); 2, Allan and Malmqvist (1989); 3, Allen (1940); 4, Allen and Claussen (1960); 5, Angradi and Griffith (1990); 6, Bailey et al. (1975); 7, Bannon and Ringler (1986); 8, Bisson (1978); 9, Bozek et al. (1994); 10, Brodeur (1989); 11, Brodeur (1991); 12, Bryan and Larkin (1972); 13, Cadwallader and Eden (1981); 14, Campbell (1979); 15, Cavalli and Chappaz (1996); 16, Christie et al. (1987); 17, Craddock et al. (1976); 18, Cunjak (1992) and R.A. Cunjak, unpublished data, University of New Brunswick, Fredericton, N.B.; 19, Eck and Wells (1986); 20, Efford and Tsumura (1973); 21, Eggers (1982); 22, Egglishaw (1967); 23, Elliott (1967); 24, Elrod and O' Gorman (1991); 25, Emmett et al. (1986); 26, Feller and Kaczynski (1975); 27, Field-Dodgson (1988); 28, Frost and Smyly (1952); 29, Furukawa-Tanaka (1985); 30, Garman and Nielsen (1982); 31, Gerke and Kaczynski (1972); 32, Glova and Sagar (1991); 33, Glova et al. (1992); 34, Godin (1981); 35, Healey (1991); 36, Hodgson et al. (1991); 37, Hubert and Rhodes (1992); 38, Hubert et al. (1994); 39, Hudson et al. (1995); 40, Hunt and Jones (1972); 41, Irie (1987); 42, Irvine and Northcote (1982); 43, Johnson and Ringler (1981); 44, Kaczynski et al. (1973); 45, Keeley and Grant (1997); 46, Kjelson et al. (1982); 47, Kocik and Taylor (1987); 48, L'Abée-Lund et al. (1992); 49, LaVoie and Hubert (1994); 50, Magnan and Fitzgerald (1982); 51, Malmquist et al. (1992); 52, Martin (1970); 53, Mason (1974); 54, McLaughlin et al. (1994); 55, McLennan and MacMillan (1984); 56, Merkel (1957); 57, Metz (1974); 58, Miller and Holey (1992); 59, Moore and Moore (1974); 60, Morgan et al. (1986); 61, Mundie (1971); 62, Murphy et al. (1987); 63, Nagy (1976); 64, Nakano (1995); 65, Northcote et al. (1979); 66, O'Donoghue and Boyd (1932); 67, Olson et al. (1988); 68, Parkinson et al. (1989); 69, Pearcy et al. (1990); 70, Peterson et al. (1982); 71, Pidgeon (1981); 72, Reid (1961); 73, Reimchen (1990); 74, Ricker (1937); 75, Rondorf et al. (1990); 76, Rose (1986); 77, Sagar and Eldon (1983); 78, Sagar and Glova (1987); 79, Schneindervin and Hubert (1987); 80, Shnavevich and Moshuk (1957) (cited from Holcík et al. 1988); 81, L. Sulek, J.S. Baxter, and E.B. Taylor, unpublished data, University of British Columbia, Vancouver, B.C.; 82, Swedberg and Peck (1984); 83, Tabor et al. (1996); 84, Thonney and Gibson (1989); 85, Tippets and Moyle (1978); 86, Trippel and Beamish (1993); 87, Van Vliet and Qadri (1970); 88, Van Oosten and Deason (1937); 89, Wilzbach et al. (1986); 90, Witkowski and Kowalewski (1984); 91, Wolf et al. (1983); 92, Wurtsbaugh et al. (1975); 93, Yule and Luecke (1993).

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Fig. 1. Mean (+SE) percentage of the number of prey in stomachs of salmonid fishes in relation to body size (10-cm size intervals) for populations from streams (*a*), lakes (*b*), and oceans (*c*). Percent composition according to different taxonomic categories is defined by the following bar types: solid, fish; open, insects; hatched, crustaceans; cross-hatched, other (mollusks, plants, amphibians, mammals, and detritus combined). Data are compiled from 81 different studies. Numbers represent sample sizes (i.e., the number of studies contributing a datum for that size category and habitat type).

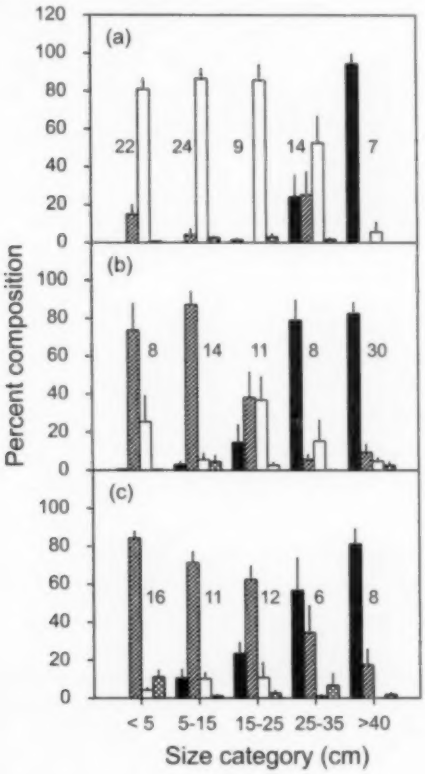
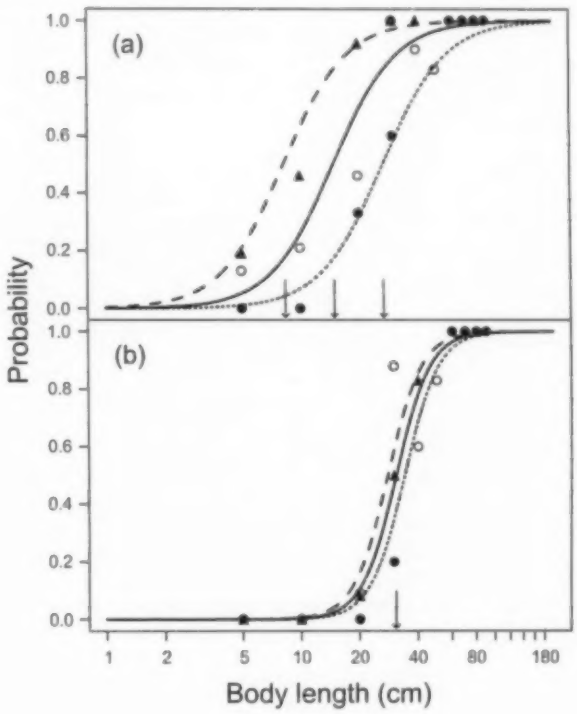


Fig. 2. (*a*) The probability of observing any fish in the diet of salmonid fishes according to body length and habitat. The broken line and solid triangles represent fish from oceans, the solid line and open circles represent fish from lakes, and the dotted line and solid circles represent fish from streams. Symbols represent the mean proportion for each 10-cm size interval. The logistic regression for any fish in the diet is logit(p) = 6.13 $\log_{10}$ salmonid length (cm) + 1.57 habitat – 10.32, n = 196, $P < 0.0001$, where habitat is 1 for streams, 2 for lakes, and 3 for oceans. Arrows indicate the body length when half the fish will contain some fish in their stomachs: 8.4 cm in oceans, 15.0 cm in lakes, and 26.9 cm in streams. (*b*) The logistic regression for a dominant percentage (≥60%) of fish in the diet is logit(p) = 11.25 $\log_{10}$ salmonid length (cm) – 17.81, n = 196, $P < 0.0001$. Symbols as in *a*. Half the fish will contain primarily fish in their stomachs at a body length of 30.8 cm.



numerical frequency of prey items (Table 1). The precision of prey taxonomy varied widely among studies. Important differences in prey composition were evident between the three habitats, even using our simple categories of prey type. Insects were the dominant type of invertebrate prey in streams, whereas crustaceans were the dominant type in lakes and oceans (Fig. 1). When paired by size category, the percentage of insects consumed was greater in streams than in either lakes (paired t = 3.42, n = 5, P = 0.027) or oceans (paired t = 4.16, n = 5, P = 0.014). The percentage of crustaceans consumed by salmonids was greater in oceans than in streams (paired t = 3.45, n = 5, P = 0.026) but did not differ between oceans and lakes (paired t = 1.42, n = 5, P = 0.23) or between lakes and streams (paired t = 1.89, n = 5, P = 0.13). The percentage of fish consumed did not differ significantly among the three habitats (analysis of variance (ANOVA), $F_{2,12}$ = 0.14, P = 0.87).

In streams, the percentage of insects in the diet decreased with increasing fish size (r = –0.64, n = 72, $P < 0.0001$; Fig. 1*a*) and the percentage of crustaceans in the diet was not related to fish length (r = –0.10, n = 72, P = 0.39; Fig. 1*a*). The percentage of crustaceans in the diet decreased as fish size increased for both lakes (r = –0.70, n = 71, $P < 0.0001$; Fig. 1*b*) and oceans (r = –0.67, n = 53, $P < 0.0001$; Fig. 1*c*). The percentage of insects in the diet also decreased with increasing fish length both in lakes (r = –0.25, n = 71, P = 0.040; Fig. 1*b*) and in oceans (r = –0.30, n = 53, P = 0.027; Fig. 1*c*).

The percentage of fish in the diet increased with fish length for streams (r = 0.87, n = 72, $P < 0.0001$), lakes (r = 0.79, n = 71, $P < 0.0001$), and oceans (r = 0.78, n = 53, $P < 0.0001$). The probability of observing any fish in the diet increased with increasing fish length (χ^2 = 51.92, n = 196, $P < 0.0001$). However, fish appeared in the diet at a

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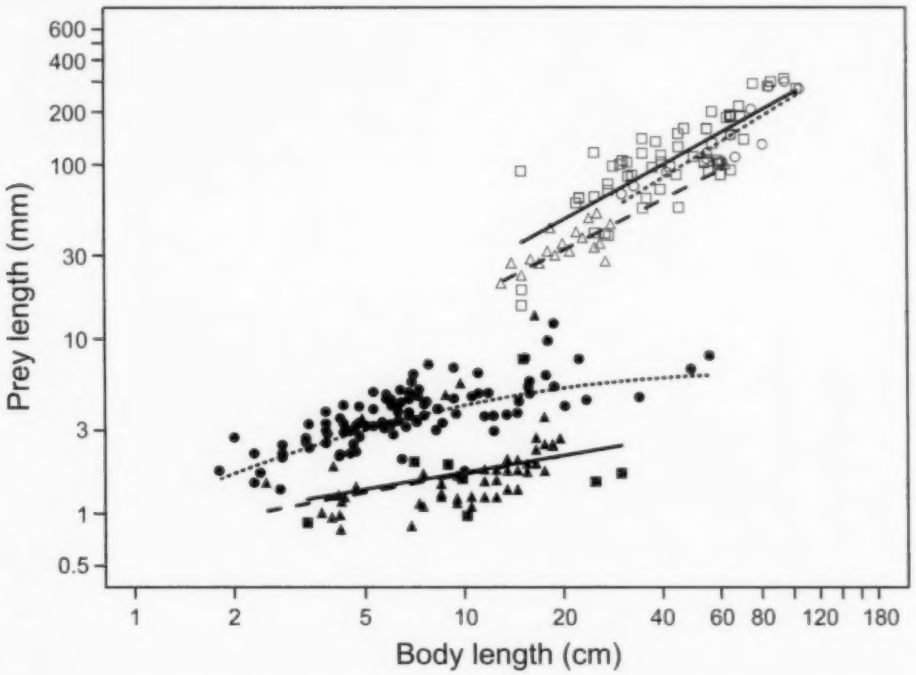
Fig. 3. The relationships between the size of salmonid fishes and their prey. The dotted line and solid circles represent stream fish feeding primarily on invertebrates: $\log_{10}$ prey length (mm) = 0.85 $\log_{10}$ predator length (cm) – 0.23 $\log_{10}$ predator length^2 (cm) + 0.033 (n = 100, $P < 0.0001$, $R^2 = 0.55$). The solid line and solid squares represent lake fish feeding primarily on invertebrates: $\log_{10}$ prey length (mm) = 0.32 $\log_{10}$ predator length (cm) – 0.089 (n = 8, $P = 0.40$, $r^2 = 0.12$). The broken line and solid triangles represent ocean fish feeding primarily on invertebrates: $\log_{10}$ prey length (mm) = 0.35 $\log_{10}$ predator length (cm) – 0.13 (n = 48, $P = 0.0011$, $r^2 = 0.21$). The dotted line and open circles represent stream piscivores: $\log_{10}$ prey length (mm) = 1.17 $\log_{10}$ predator length (cm) – 0.051 (n = 13, $P < 0.0001$, $r^2 = 0.81$). The solid line and open squares represent lake piscivores: $\log_{10}$ prey length (mm) = 1.04 $\log_{10}$ predator length (cm) – 0.33 (n = 60, $P < 0.0001$, $r^2 = 0.65$). The broken line and open triangles represent ocean piscivores: $\log_{10}$ prey length (mm) = 0.96 $\log_{10}$ predator length (cm) – 0.25 (n = 20, $P < 0.0001$, $r^2 = 0.66$).



smaller body size in oceans than in lakes, and in lakes than in streams ($\chi^2 = 21.031$, n = 196, $P < 0.0001$; Fig. 2*a*). The probability of observing a dominant proportion (≥60%) of fish in the diet also increased with fish size ($\chi^2 = 33.74$, n = 196, $P < 0.0001$; Fig. 2*b*) but did not differ among habitats ($\chi^2 = 1.51$, n = 196, $P = 0.22$).

Prey size

Sixty-one studies provided data for both prey size and fish size (Table 1). In general, the average size of food items in the diet increased with increasing body size of the fish (r = 0.81, n = 220, $P < 0.0001$; Fig. 3). There was an obvious difference, however, between the sizes of prey eaten by salmonids specializing on invertebrates versus fish. Piscivorous salmonids ate prey that were at least one order of magnitude longer than invertebrate-eating salmonids (Fig. 3).

In addition, there were differences among habitats in the size of invertebrate-prey consumed. The size of invertebrate prey increased with fish size in all three habitats. This increase was linear in both lakes and oceans (Fig. 3) and did not differ in either slope (analysis of covariance (ANCOVA), $F_{1,55} = 0.02$, $P = 0.89$) or elevation (ANCOVA, $F_{1,55} = 0.01$, $P = 0.91$). The common regression equation for all salmonids in lakes and oceans was $\log_{10}$ prey length (mm) = 0.35 $\log_{10}$ predator length (cm) – 0.12 (n = 56, $P = 0.0009$, $r^2 = 0.19$). In streams, however, the increase in invertebrate prey size with increasing fish size followed a quadratic relationship; there appeared to be an asymptotic average prey length of about 6.5 mm (Fig. 3). Invertebrates eaten by stream salmonids were approximately three times longer than those consumed by fish of equivalent size in lakes and oceans. For example, 99 of 100 data points for stream fish were above the regression for lakes and oceans (binomial test, $P < 0.001$).

For salmonids that were primarily piscivores, prey size increased linearly with body length in all three habitats (open symbols, Fig. 3). While the three regressions for piscivores did not differ significantly in slope (ANCOVA, $F_{2,92} = 0.20$, $P = 0.82$), they did differ in elevation (ANCOVA, $F_{2,92} = 9.05$, $P = 0.0003$). Piscivores in streams and lakes did not differ significantly in the size of prey eaten (adjusted least-squares means, $P = 0.23$) but piscivores in oceans ate smaller prey than those in lakes (adjusted least-squares means, $P < 0.0001$) and streams (adjusted least-squares means, $P = 0.049$).

Interspecific comparisons

To test for species-specific differences in prey size, we calculated separate regressions for each species by habitat by prey type (fish or invertebrate) combination with at least 10 observations. Of the 10 separate regressions for nine dif-

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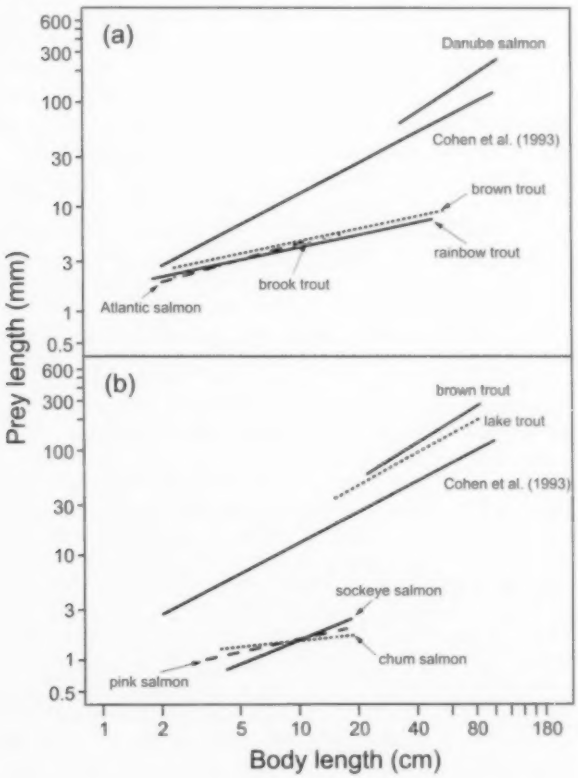
Table 2. Linear regressions of $\log_{10}$ prey size (mm) versus $\log_{10}$ body length (cm) for several salmonid fishes by either an ordinary least-squares (OLS) or a reduced major-axis (RMA) regression technique.

			OLS regression					RMA regression
Species[a]	Habitat	Prey type	Slope (SE)	Intercept (SE)	n	r^2	P	Slope
Atlantic salmon	Stream	Invertebrates	0.53 (0.11)	0.12 (0.091)	17	0.61	0.0002	0.68
Brook trout	Stream	Invertebrates	0.48 (0.052)	0.15 (0.036)	13	0.90	0.0001	0.51
Brown trout	Stream	Invertebrates	0.40 (0.064)	–0.27 (0.057)	33	0.56	0.0001	0.53
Rainbow trout	Stream	Invertebrates	0.40 (0.063)	0.21 (0.062)	20	0.69	0.0001	0.48
Chum salmon	Ocean	Invertebrates	0.19 (0.14)	–0.008 (0.14)	20	0.09	0.19	—
Pink salmon	Ocean	Invertebrates	0.76 (0.11)	–0.57 (0.12)	13	0.81	0.0001	0.84
Sockeye salmon	Lake	Invertebrates	0.41 (0.17)	–0.21 (0.18)	15	0.31	0.030	0.74
Danube salmon	Stream	Fish	1.23 (0.21)	–0.087 (0.38)	12	0.78	0.0002	1.39
Brown trout	Lake	Fish	1.17 (0.16)	0.20 (0.26)	13	0.83	0.0001	1.28
Lake trout	Lake	Fish	1.04 (0.13)	0.32 (0.21)	41	0.64	0.0001	1.30

[a]See Table 1 for scientific names.

Fig. 4. Comparative allometry of prey size versus predator size for several species of salmonid fishes in streams (*a*) or lakes and oceans (*b*) and in comparison with a general equation for animal predators from Cohen et al. (1993). See Table 2 for salmonid regression equations.



ferent species, prey size increased significantly with fish size in all cases, except for chum salmon (*Oncorhynchus keta*) (Table 2). Within a habitat type or prey type, there was little evidence of differences among species in prey size (Figs. 4*a* and 4*b*; Table 2). Using ANCOVA, there were no significant differences in the prey-size versus predator-size relationships for salmonid fishes eating invertebrates in streams (slope, $F_{3,74} = 0.44$, $P = 0.73$; elevation, $F_{3,74} = 2.57$, $P = 0.061$), invertebrates in lakes (slope, $F_{2,42} = 2.95$, $P = 0.063$; elevation, $F_{2,42} = 0.02$, $P = 0.98$), or fish in all habitats (slope, $F_{2,59} = 2.15$, $P = 0.13$; elevation, $F_{2,59} = 2.10$, $P = 0.13$).

We also compared our data with Cohen et al.'s (1993) general relationship for prey length versus predator length for a variety of terrestrial and aquatic habitats (RMA regression: $\log_{10}$ prey length (cm) = 0.982 $\log_{10}$ predator length (cm) – 0.864; 95% confidence interval (CI) for the slope = 0.901–1.063). All nine significant slopes for the species regressions in Table 2 differed significantly from Cohen et al.'s (1993) relationship; that is, RMA regression slopes in Table 2 were outside their 95% CI. The slopes of the allometric prey size relationship for invertebrate-eating salmonids were lower than expected by Cohen et al. (1993), whereas the opposite trend was observed for piscivorous salmonids. The average length of salmonids in our study (geometric mean = 13.67 cm) did not differ significantly from the mean in Cohen et al. (1993) (geometric mean = 9.90 cm, $t = 0.44$, df = 702, $P > 0.5$). Invertebrate prey were smaller than expected (binomial test, $P < 0.001$) by Cohen et al. (1993), whereas fish prey were larger than expected (binomial test, $P < 0.001$; Fig. 5).

Morphological constraints and prey size

The range of mean prey size eaten was generally within the hypothesized constraints of gill-raker spacing and mouth size. Only 10 of 220 observations were smaller than the minimum predicted by Wańkowski's (1979) equation of gill-raker spacing for Atlantic salmon, whereas only two of 220 observations were larger than the maximum predicted by Damsgård's (1995) equation for mouth gape in brown trout (Fig. 5). Piscivorous salmonids ate relatively large prey that approached the maximum predicted by Damsgård's (1995) equation (Fig. 5). In contrast, invertebrates eaten in lakes and oceans approached the minimum size predicted by gill-raker spacing. Salmonids in streams ate invertebrates that were in the middle of the size spectrum.

Discussion

Our synthesis of the published literature indicated that salmonid fishes in all three habitats exhibited an ontogenetic

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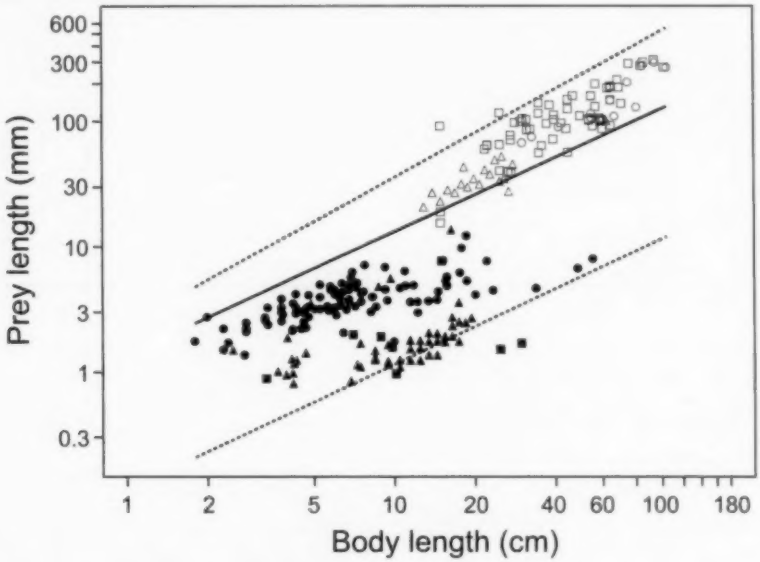
Fig. 5. Mean prey length in relation to salmonid predator length; symbols are as depicted in Fig. 3. The upper dotted line represents the maximum prey size predicted by Damsgård's (1995) gape-limitation equation for brown trout and the solid line represents a general equation for animal predators from Cohen et al. (1993). The lower dotted line represents the minimum prey size predicted by Wańkowski's (1979) gill raker spacing equation for Atlantic salmon.



niche shift from feeding on invertebrates when small to feeding on fish when large. Before switching to fish, small salmonids appeared to feed almost exclusively on insects in streams and crustaceans in oceans. The initial feeding niche in lakes was intermediate between streams and oceans. The difference between streams and oceans was not surprising, because insects are abundant in fresh water but are almost absent from marine habitats where they are replaced by crustaceans (Margulis and Schwartz 1998). While crustaceans dominated the diet of small salmonids in lakes, there was a hint of an intermediate feeding niche in which salmonids fed equally on crustaceans and insects before switching to piscivory. These ontogenetic shifts in diet presumably occur as salmonids attempt to eat more energetically profitable prey as they grow larger. As body size increases, optimal prey size also increases as the handling time for larger prey decreases (Wańkowski and Thorpe 1979; Bannon and Ringler 1986). Such ontogenetic shifts have been observed within many populations (Brodeur 1991; L'Abée-Lund et al. 1992).

In contrast with the predictions of the prey-size hypothesis, invertebrate prey were larger in streams than in lakes and oceans and fish prey were smaller in oceans than in streams and lakes. However, salmonids began feeding on fish at a smaller size in oceans than in lakes, and in lakes than in streams, a result that is consistent with the prey-size hypothesis. These habitat differences in the onset of piscivory were likely the result of the availability of suitable forage fish. Because we used the average data from each study, the actual size at which salmonids begin eating fish may be even smaller. Interestingly, salmonids became primarily piscivorous at about 31 cm in length, regardless of habitat type. Perhaps a greater abundance and a greater range of size of potential forage fish in oceans allow salmonids to begin feeding on fish at a small size and hence grow quickly. By the time a salmonid is 31 cm in length, invertebrates may no longer be very profitable prey, regardless of habitat type.

The relatively slow growth of salmonids in streams (Bachman 1982; Hutchings 1986) cannot be due to the small size of invertebrate prey, but may be related to the lack of a reliable supply of forage fish. A 15- to 20-cm fish is already eating the largest insects that are available in the drift, and will likely begin a steady decline or cessation in growth at about this size unless forage fish are available (Pidgeon 1981; Bannon and Ringler 1986). On average, stream salmonids only begin eating fish when 27 cm long. When stream salmonids are able to switch to piscivory, they can grow to over 50 cm in length (Holcík et al. 1988).

The faster growth of salmonids in lakes than in streams is not because the invertebrate prey are larger. Faster growth in lakes may be due to either the higher productivity of invertebrates or the opportunity to begin eating fish at a smaller size (about 15 cm). Fast growth to attain a large body size in lake salmonids requires large prey, typically fish, whether they occur naturally (Campbell 1979; Trippel and Beamish 1993) or are introduced (Rasmussen et al. 1990). Planktivorous lake trout eventually cease growth at a small size, even though prey are apparently abundant (Konkle and Sprules 1986). Similarly, the faster growth of salmonids in oceans than in fresh water is not related to the size of invertebrate prey, but may be related to either the higher productivity of the habitat (Gross et al. 1988) or the opportunity to begin eating fish at a small size (at about 8 cm in length).

In general, habitat type had a greater effect on the size and composition of the diet than did taxonomy. Few differences between species in the allometry of prey size were evident in our broad-scale analysis. The diet of a typical salmonid may be broadly set by its morphology and the availability of prey. The narrow spacing of the gill rakers allows them to eat rela-

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tively small prey compared with most predators of equivalent size (Cohen et al. 1993). Compared with other piscivores, salmonids have small mouths and begin eating fish at a larger size and later age (Mittelbach and Persson 1998). As piscivores, salmonids appear to be gape-limited and increasingly eat larger fish as they grow. Despite their relatively late switch to piscivory, salmonids eat larger prey than expected for a predator of equivalent size (Cohen et al. 1993) and grow larger than expected compared with other piscivores (Mittelbach and Persson 1998).

In summary, our analysis suggests that different species of salmonid fishes represent relatively little variation about a common dietary theme. We suggest that salmonid species of a similar size in a similar habitat will overlap broadly in the size and composition of their diet.

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Spatial and temporal effects of interspecific competition between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) in winter

A.J. Harwood, N.B. Metcalfe, J.D. Armstrong, and S.W. Griffiths

Abstract: Previous work has shown that juvenile stream-dwelling salmonids become predominantly nocturnal during winter by emerging from daytime refuges to feed, with several species having been shown to prefer slow-flowing water while active at night. We used seminatural stream channels, landscaped to provide a choice of water depths, and hence velocities, to test whether Atlantic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, show similar habitat preferences during winter. We also tested whether there was any spatial or temporal displacement of Atlantic salmon when in sympatry with brown trout. Nighttime observations revealed that Atlantic salmon did have a preference for slow-flowing water. However, when in direct competition with trout, salmon either remained predominantly nocturnal but occupied shallower water, or became significantly less nocturnal, spending more time active during the day than when in allopatry. These results, which were especially marked in relatively larger fish, indicate that competition between the two species for food and resources is not restricted to the summer months and may affect both the short- and long-term growth and survival of overwintering wild Atlantic salmon.

Résumé : Des travaux antérieurs ont démontré que de jeunes salmonidés habitant les eaux courantes en hiver deviennent surtout nocturnes et qu'ils quittent leurs refuges de jour pour s'alimenter la nuit; plusieurs espèces montrent alors une préférence pour les eaux plus calmes durant cette activité nocturne. Des ruisseaux artificiels, aménagés de façon à offrir un choix de profondeurs et, par conséquent, de vitesses de courant, ont servi à vérifier si des Saumons de l'Atlantique, *Salmo salar*, et des Truites brunes, *Salmo trutta*, ont des préférences d'habitat similaires en hiver. Il a aussi été possible de tester s'il s'opère un déplacement temporel ou spatial du Saumon de l'Atlantique lorsqu'il cohabite avec la Truite brune. Des observations de nuit ont révélé que le Saumon de l'Atlantique a une préférence pour les zones à courant lent. Cependant, lorsque le saumon est en compétition directe avec la truite, ou bien il demeure surtout nocturne, mais en occupant les eaux moins profondes, ou alors il passe plus de temps en activité le jour qu'il ne le ferait en l'absence des truites. Ces résultats, qui sont particulièrement nets chez les poissons relativement plus grands, indiquent que la compétition entre les deux espèces pour la nourriture et les ressources ne se limite pas aux mois d'été, mais qu'elle peut affecter la croissance à court et à long termes et la survie des Saumons de l'Atlantique sauvages pendant l'hiver.

[Traduit par la Rédaction]

Introduction

Diel activity patterns of organisms result from the need to concentrate activity at a time that optimises the balance between competition, predation risk, and food availability (Helfman 1993). For instance, individual rainbow trout, *Oncorhynchus mykiss*, and Arctic charr, *Salvelinus alpinus*, may adopt contrasting diel activity patterns, possibly as a result of subordinates attempting to attain adequate growth while avoiding high levels of aggression from more dominant fish (Alanärä and Brännäs 1997). A grazing mayfly, *Baetis* sp., was aperiodic or weakly nocturnal in a fishless stream but became more nocturnal when transferred to a trout-inhabited stream, illustrating the influence of predation risk on diel feeding (Cowan and Peckarsky 1994).

Both experimental and field studies have shown that juvenile Atlantic salmon, *Salmo salar*, undergo a temperature-dependent shift from predominantly diurnal activity above 10°C to predominantly nocturnal activity at lower temperatures (Fraser et al. 1993, 1995; Bremset 2000). Such a switch may account for seasonal changes in diel activity of not only Atlantic salmon but also brown trout, *Salmo trutta*

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(Heggenes et al. 1993), rainbow trout (Riehle and Griffith 1993), and Arctic charr (Linnér et al. 1990). This change in behaviour may be a response to their increased vulnerability in winter to diurnal endothermic predators (Metcalfe et al. 1999) that are not affected by decreased water temperatures, which cause the swimming ability and acceleration of fish to be reduced (Rimmer et al. 1985). Alternatively, the shift to nocturnal activity may be a stratagem for avoiding being trapped by anchor ice (Heggenes et al. 1993; Whalen et al. 1999) or may be a response to the pattern of drifting or benthic invertebrates (e.g., Amundsen et al. 2000).

Since salmonids are sit-and-wait predators that rely on vision, the cost of becoming more nocturnal is a decrease in the efficiency of capturing individual food items. Even on the clearest of nights, feeding efficiency is only approximately 35% of that during the day (Fraser and Metcalfe 1997). However, nocturnal foraging may decrease predation risk (expressed per unit of food intake) and therefore may have fitness benefits (Metcalfe et al. 1999). Brown trout prefer slower water currents at night than during the day (Heggenes et al. 1993), presumably to see their prey more easily. Moreover, juvenile Atlantic salmon prefer slower water currents on dark nights than on clear nights, to facilitate prey capture (Metcalfe et al. 1997). As expected, the preference of salmon to use deep slow water actively on winter nights was observed in the wild by Whalen and Parrish (1999).

A greater use of deep water in winter is likely to cause increased competition for foraging opportunities between sympatric salmon and brown trout. In summer months, juvenile trout are more aggressive than salmon of similar size (e.g., Kalleberg 1958) and therefore restrict them to less-preferred (i.e., shallower) areas of a stream (Kennedy and Strange 1986). Such interspecific competition is thought to have long-lasting effects on growth, survival, and fitness (Fausch 1998). Nonetheless, winter has been considered to be the most important period for survival of salmon parr (Cunjak et al. 1998), and interspecific interactions may be important at this time of year. However, as yet there is no information on the relative competitive abilities of trout and salmon during winter.

We conducted experiments using artificial stream channels to test three hypotheses: (1) juvenile Atlantic salmon prefer deep water in winter when living in allopatry (cf. Whalen and Parrish 1999), (2) juvenile Atlantic salmon are displaced from their preferred habitat when living in sympatry with brown trout, and (3) interspecific competition for space in winter results in shifts in diel activity patterns and (or) temporal changes in the habitat use of juvenile Atlantic salmon.

Methods

The experiment was conducted in an outdoor artificial stream (previously described in Valdimarsson et al. 1997) at the University Field Station, Rowardennan, Loch Lomondside, Scotland. The stream was 60 cm wide and formed a continuous, approximately oval shaped loop with straight sides. Fish could be observed from within this loop, since the inside walls of the channel were made from glass panels. The straight sides of the channel were each 6 m in length; however, the variable-speed impeller that pumped water around the channel occupied 2.5 m of one side. The remaining straight parts of the channel were partitioned into three 2.5-m experimental arenas, using mesh, and landscaped with fine gravel (5–25 mm in diameter) to deter fish from hiding in stream bed cavities. The gravel was landscaped to produce a smooth gradient within each arena, with shallow water (10 cm) at the upstream end and relatively deep water (45 cm) at the downstream end. The surface velocity of the water varied continuously from 0.16 $m \cdot s^{-1}$ at the upstream end to 0.03 $m \cdot s^{-1}$ at the downstream end. A 1 m long section between each experimental arena was landscaped with the contrasting gradient, to decrease turbulence and smooth the flow down the channel. The outer sides of the channel were marked every 15 cm along their length, to define 15 zones per arena (zone 1 being at the upstream end) and allow referencing of the recorded positions of the fish. Fish shelters were made from six 1-L opaque bottles that were cut in half lengthwise and embedded in the gravel at regular intervals in each of the experimental arenas, creating cavities in the gravel measuring 17 × 8.5 × 4.25 cm. These bottles were positioned with the open side against the glass wall, to allow any fish within them to be identified, a technique successfully used by Valdimarsson et al. (1997).

Water was pumped constantly from Loch Lomond and ranged in temperature from 4.3 to 6.1°C. Light conditions and photoperiod for the outdoor stream channel were the same as the ambient conditions of Loch Lomond. However, some overhead cover was provided at all water depths by placing wooden planks approximately 15 cm above the water surface, across half the width of the channel. The position of these planks was randomised with respect to the inside and outside walls of the stream channel. This allowed comparison of the amount of time spent under overhead cover and out of cover. Food was provided via a belt feeder, which dropped pelleted food at a trickle rate at the upstream end of each arena. Fish were provided with 0.5% of their total body weight in food per day, an amount chosen so growth would be minimal but fish would not lose condition during the experimental trials. This pelleted food was supplemented by the low level of zooplankton found in the water pumped from Loch Lomond.

Two treatments were used to test the effects of trout on the behaviour of salmon: allopatric (four salmon) and sympatric (two salmon with two trout). Data collected from the salmon in allopatry would reveal whether salmon prefer areas of deep slow-moving water or areas of shallow fast-flowing water, while the sympatric treatment would reveal whether habitat use changes under conditions of interspecific competition. Fish in both treatments were size-matched to reduce any confounding effects of large size differences. Both treatments were run simultaneously in different arenas of the stream channel, to prevent any confounding effects of temperature or time of year influencing the results.

The fish used were wild-caught salmon (fork length = 109.4 ± 1.83 mm (mean ± standard error (SE)); weight = 13.42 ± 0.65 g) and trout (fork length = 100.7 ± 2.77 mm; weight = 9.87 ± 0.90 g) from the River Blane, a tributary of the River Endrick that flows into Loch Lomond. Fish were individually marked on their dorsal and caudal fins using small injections of alcian blue dye, after anaesthetisation using benzocaine. Fish were then allowed to reside in the experimental arenas for a period of 72 h before observations began.

The observations of each group of fish were made on four consecutive dates. Each night of observation lasted 7 h and covered the day–night transition at either dusk or dawn. During this observation period, the position of each fish was recorded every 30 min by scanning briefly with a flashlight, a method thought to minimise disturbance of the fish (see Heggenes et al. 1993). Data collected during each scan observation consisted of the position of each fish in the water column (either resting on the bottom or swimming in the water column), the number of the zone in which the fish was located (1–15, which was a relative measure of water depth, and hence velocity), and whether or not the fish was under overhead cover. The use of shelters was also recorded, to determine the

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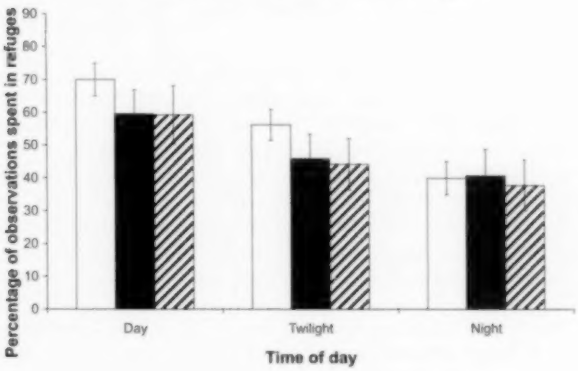
Table 1. Summary of results from a repeated measures ANOVA for the effect of time of day on behavioural responses; in all cases, multivariate significance tests were used, as Mauchly's test of sphericity was significant.

Source of variation	Proportion of time spent in refuges			Proportion of time spent on substrate while not in refuges			Proportion of time spent under overhead cover while not in refuges		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Within subjects									
Time of day	2	16.70	<0.001	2	3.70	0.038	2	0.38	0.688
Error	60			27			27		
Between subjects									
Category of fish	2	0.74	0.481	2	8.01	0.002	2	0.60	0.558
Error	61			28			28		
Time of day × category of fish	4	0.60	0.662	4	1.93	0.119	4	0.30	0.874
Error	122			56			56		

Fig. 1. Graph of the percentage (±SEM) of observations spent in refuges at various times during a 24-h period for salmon in allopatry (open bars), salmon in sympatry (filled bars), and trout in sympatry (shaded bars). Percentages are arcsine transformed, thus fish with a value of 90.0% never left the refuges.



frequency with which fish sought refuge; light intensity and water temperature were also noted. Light intensity was measured using a Skye Instruments SKL 300 photometer (range 0.01–2000 lx) and recorded as the mean of two measurements made just above the overhead cover provided in the middle of the straight section on either side of the channel. Light intensity readings were used to split observations into three categories, day (≥100 lx), twilight (between 0.02 and 100 lx), and night (≤0.02 lx). Water temperature was measured using a digital thermometer (Oregon Scientific EM913, resolution 1°C) placed permanently in the stream. After the data for each group were collected, the fish were removed and different fish placed in the test arena. Both treatments were replicated eight times; all fish were used only once. The experiments were carried out between 16 January and 4 March 1999.

Data analysis and statistical treatment

For the purpose of comparing treatments, the data were split into three categories: salmon in allopatry, salmon in sympatry (with trout), and trout in sympatry (with salmon), with data for the latter two categories coming from the one sympatric treatment. To evaluate the effect of body size on behaviour while standardising for size differences between trials, the relative body length (body length as a proportion of the body length of the largest fish in each group of four fish) was calculated for each fish. All percent data were normalised by arcsine transformation prior to use in parametric tests, and all quoted probabilities are for two-tailed tests of significance.

Repeated measures analysis of variance (ANOVA) was used to test the null hypotheses that time of day (within-subject effect) and category of fish (between-subject effect) had no effect on response variables (proportion of observations when in refuges or on substrate or under overhead cover while not in refuges).

To compare the timing of activity between treatments, the percentage of activity (i.e., time out of shelters) that occurred by night was determined for each fish. This was calculated using the formula:

$$100 \times E_n/(E_n + E_d)$$

where E_n is the percentage of nighttime and twilight observations during which a fish was out of shelter and E_d is the percentage of daytime observations during which a fish was out of shelter (Fraser et al. 1995). This was termed the nocturnal index and ranged from 0% (the fish was only observed out of shelter during the day) to 100% (the fish was only observed out at night). To compare between treatments, a mean value for each category of fish was calculated for each replicate group.

Results

Time of day effects on the use of cover

As expected, there was a significant difference in the proportion of time that both salmon and trout were observed to spend in refuges during day, twilight, and night (Table 1; Fig. 1). This difference represented a significant decrease in the use of shelters from daytime through twilight to night (linear term of polynomial contrasts: $F_{1,61} = 28.63$, $P < 0.001$). However, there was no significant difference in this time effect between categories of fish (Table 1; Fig. 1), indicating that both salmon and trout became more active at night. There was a significant difference in the proportion of time that both salmon and trout were observed to spend on the substrate while not sheltering during all three time periods (Table 1; Fig. 2). This difference represented a significant increase in the amount of time spent on the substrate from daytime through twilight to night (linear term of polynomial contrasts: $F_{1,28} = 7.20$, $P = 0.012$). There was also a significant difference between the species, with salmon observed on the substrate more often than trout (Table 1; Fig. 2). There was no time of day or species effect on the proportion of time observed under overhead cover (Table 1).

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Fig. 2. Graph of the percentage (±SEM) of observations spent holding position on the substrate while not in refuges for salmon in allopatry (open bars), salmon in sympatry (filled bars), and trout in sympatry (shaded bars). Percentages are arcsine transformed, thus fish with a value of 90.0% were always found resting on the substrate.

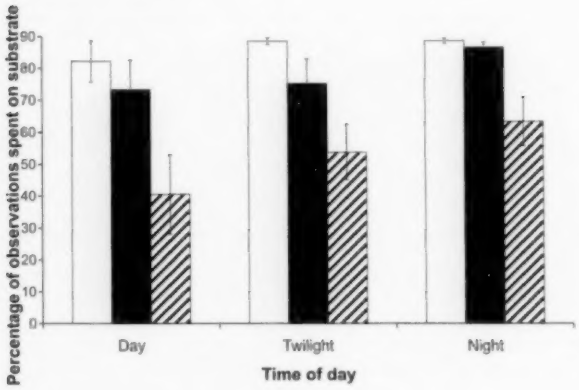

Fig. 3. The average position of each fish while out of shelter during the night plotted against relative length for salmon in allopatry (open circles, dotted line), salmon in sympatry (shaded squares, broken line), and trout in sympatry (filled triangles, solid line). Average position is calculated by averaging zone use during the night. Zone 15 is the deepest and thus has the slowest-flowing water, while zone 1 is the shallowest and thus has the fastest-flowing water. Similar trends were witnessed during daylight and twilight observations, however these are not presented for the sake of clarity.

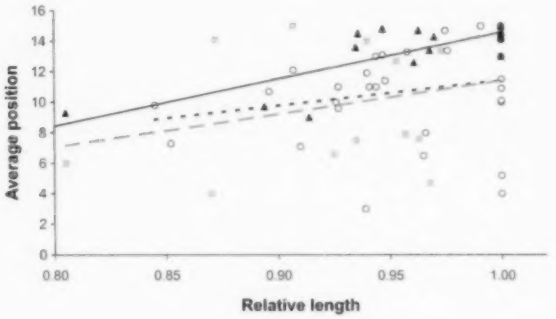

Table 2. Summary of the results from a stepwise multiple regression for the relative influence of light intensity, time of year (Julian date), and temperature on the use of refuges.

Variable	Order in which entered	t	P
Light	1	8.26	<0.001
Temperature	2	9.62	<0.001
Day	3	7.16	<0.001

Note: Overall, $r^2 = 0.333$, $F_{3,345} = 58.91$, $P < 0.001$.

Influence of light intensity, temperature, and time of year on refuge use

To test for the relative influence of light intensity, time of year (Julian date), and temperature on the use of refuges, a stepwise multiple regression was performed on the (arcsine-transformed) percentage of fish hiding at each observation. Owing to the fact that no significant difference was found in the proportion of time spent in shelter between categories of fish (Table 1), this analysis was carried out on the overall percentages of both species combined that were observed in the refuges. It revealed that light intensity was the most important single explanatory variable, and its effect was independent of that of date and temperature (see Table 2).

Effect of body size and treatment on the depth of water used

To compare the influence of absolute and relative body size on the position adopted by fish, a stepwise multiple regression was carried out on the mean zone in which a given fish was observed when out of shelter, irrespective of time of day or species. This illustrated that relative length was more important in predicting the position of fish than absolute length (stepwise multiple regression: absolute length not included in model: model, $r^2 = 0.117$, $F_{1,142} = 19.94$, $P < 0.001$). Therefore, relative length was used as the measure of body size in all subsequent analyses.

To test both our first hypothesis, that salmon prefer deep water, and our second hypothesis, that they are displaced spatially by trout, we investigated the depth of water used by individual fish. For each fish, an indication of the depth of water used was calculated separately for day, twilight, and night by averaging zone use during each of these periods over the 4 days. The effect of treatment on distribution patterns was then analysed separately for daytime, twilight, and nighttime observations, using analysis of covariance (ANCOVA), with fish category (allopatric salmon, sympatric salmon, or sympatric trout) as the factor and relative body length as the covariate. There was a significant effect of relative body length on average position during the day (ANCOVA: effect of length, $F_{1,30} = 7.98$, $P = 0.008$), during twilight ($F_{1,46} = 6.61$, $P = 0.013$), and during the night ($F_{1,56} = 9.18$, $P = 0.004$), with fish of greater length relative to the other fish in their replicate group being found in deeper water (see Fig. 3). The tendency for salmon to be found mostly in the deeper parts of the stream channels suggests that they, as well as trout, prefer deep water, thus supporting our first hypothesis. During the night there was also a significant effect of category of fish on average position (ANCOVA: comparison of regression slopes, $F_{2,54} = 0.37$, NS; comparison of regression elevations, $F_{2,56} = 4.07$, $P = 0.022$), with trout being found in deeper water than salmon of equivalent size in both allopatry and sympatry (see Fig. 3). There was, however, no significant difference in the depth of water used by salmon in allopatry and salmon in sympatry, thus providing no support for our second hypothesis.

Nocturnal versus diurnal activity

We used the calculated nocturnal index to test our third hypothesis, that interspecific competition results in a shift in diel activity and (or) temporal changes in habitat use by salmon. There was no significant difference in the nocturnal index (arcsine-transformed) between salmon and trout when

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Fig. 4. Percentage (±SEM) of nocturnal activity (where nocturnal activity is nocturnal activity/overall activity) for each treatment. Percentages are arcsine transformed, therefore 90% nocturnal activity represents fish only active at night, whereas 0% nocturnal activity represents fish active only during the day.

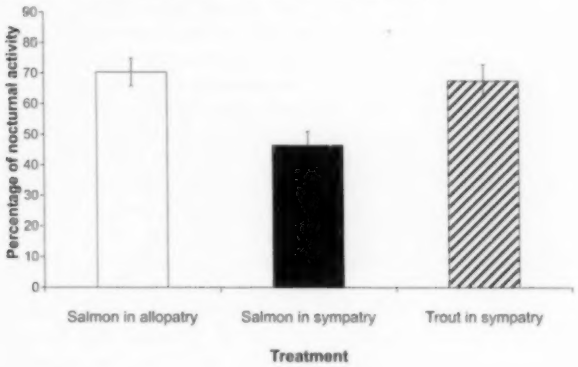
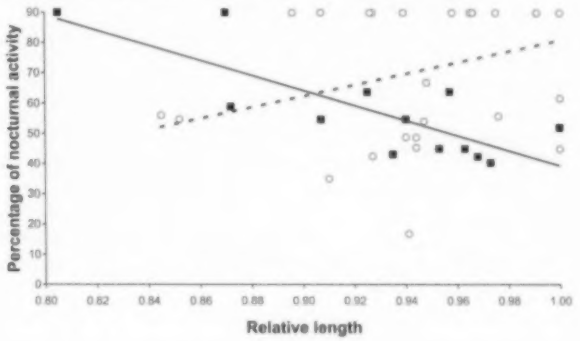


Fig. 5. The percentage (±SEM) of nocturnal activity plotted against relative length for salmon in allopatry (open circles, dotted line) and salmon in sympatry (filled squares, solid line). Percentages are arcsine-transformed, therefore 90% nocturnal activity represents fish only active at night, whereas 0% nocturnal activity represents fish active only during the day.



the two species were in sympatry (paired sample t test, $t_7 = 1.50$, NS; see Fig. 4). However, there was a significant difference in the nocturnal index between salmon in allopatry and salmon in sympatry (independent sample t test, $t_{14} = 2.39$, $P = 0.031$), with salmon in allopatry being more nocturnal (see Fig. 4). This supports our hypothesis that salmon undergo a shift in diel activity when they switch from allopatric to sympatric conditions. To determine the influence of body size on nocturnal versus diurnal activity for salmon in the two treatments, ANCOVA was performed on the nocturnal index with treatment (allopatry or sympatry) as the factor and relative size as the covariate. This revealed a significant difference in the diel activity pattern of salmon of different lengths in the two treatments, with the largest salmon in a replicate group being the most nocturnal in allopatry but the least nocturnal in sympatry (ANCOVA: comparison of regression slopes, $F_{1,40} = 11.96$, $P = 0.001$; see Fig. 5).

Nearest-neighbour distances

To gain an insight into nearest-neighbour distances, the occasions when fish occurred in the same or adjacent zones were examined. Out of a total of 245 scan samples of the three test arenas at night, fish occurred in the same or adjacent zone on 83 occasions in allopatry and 125 occasions in sympatry. A χ^2 goodness-of-fit test revealed that this represented a significant difference between the treatments ($\chi^2 = 8.48$, df = 1, $p < 0.01$), with fish more likely to be in close proximity to each other in sympatry than in allopatry. Interestingly, salmon in allopatry were in close proximity on 83 occasions, whereas in sympatry, salmon only occurred in proximity with other salmon on two occasions, with trout being found in proximity with other trout on 55 occasions. A χ^2 test revealed that this represented a significant difference between the two species ($\chi^2 = 49.28$, df = 1, $p < 0.001$).

Discussion

Habitat preferences and activity patterns

Our first hypothesis, that juvenile salmon in winter prefer deep water, was supported, with salmon preferring the deepest, and thus slowest-flowing water that was available. This preference was supported by our observation of relatively larger fish holding deep–slow positions more frequently than relatively smaller fish, coupled with the fact that, at this stage in the life cycle of both salmon and trout, dominant fish are larger than subordinates (Huntingford et al. 1990). Although this study does not differentiate between selection for water depth and selection for water velocity, previous studies of both Atlantic salmon (Whalen and Parrish 1999) and brown trout (Cunjak and Power 1986; Mäki-Petäys et al. 1997) in the wild have shown a stronger selection for water velocity than for water depth in winter. Therefore, the habitat preferences exhibited by Atlantic salmon could be considered to be a preference for slow-flowing water, occurring in areas of deep water, rather than a selection for deep water. A comparison of the average positions of brown trout in sympatry and Atlantic salmon in allopatry indicates that the trout had a stronger preference for deeper and (or) slow-flowing water, but that their preferred microhabitat overlapped that of the salmon. Rainbow trout (Riehle and Griffith 1993) and coho salmon, *Oncorhynchus kisutch* (Nickelson et al. 1992), also preferred slow-flowing water in winter. Similarly, coho salmon preferred deep pools that offered protection from fast-flowing water and turbulence over deep pools that offered no shelter from high water velocities (Nickelson et al. 1992).

A comparison of the positions adopted by salmon in allopatry and sympatry indicates that salmon were not significantly displaced from this preferred microhabitat by trout, thus providing no support for our second hypothesis. However, there is evidence of competition between the two species, with the data supporting our third hypothesis of a shift in the diel activity of Atlantic salmon caused by the presence of brown trout. The significant shift towards more daytime activity exhibited by salmon in sympatry, as opposed to salmon in allopatry, is evidence of the dominance of brown trout over Atlantic salmon. In allopatry, the largest salmon were the most nocturnal and were found in the deepest water; that they occupied this preferred spatial and temporal

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niche suggests that they are the dominant fish. Furthermore, their lack of daytime activity suggests that, under allopatric conditions, dominant salmon obtained enough food during the night to avoid risky daytime feeding. However, the presence of trout may have prevented these largest salmon from effectively feeding in deep water at night, causing them to become more active during the day. This is in accordance with earlier studies in which salmon were shown to become more diurnal when food availability was reduced or nutritional requirements were increased (see Metcalfe et al. 1998, 1999). This result is also analogous to that found by Alanärä and Brännäs (1997), who observed individual rainbow trout and Arctic charr adopting contrasting diel activity patterns, apparently to reduce competition in accessing food. The similarity in the amount of time salmon and trout were observed to spend in refuges suggests that the two species have rather similar time budgets and feeding rates in relation to their nutritional needs.

Aggregations of fish and habitat partitioning

Previous studies of nocturnal activity during winter have witnessed no aggressive interactions between conspecifics of either Atlantic salmon (Whalen and Parrish 1999) or brown trout (Heggenes et al. 1993). Other studies have concluded that aggregations of trout in pools illustrate a cessation of territorial behaviour during the winter (Cunjak and Power 1986). However, the cessation of territorial behaviour in pools has also been witnessed at other times of year (Bremset and Berg 1997) and may not be especially associated with winter. The lack of aggression in winter noted by Heggenes et al. (1993) was interpreted as an indication of a reduction in the need to feed, and hence maintain territorial boundaries. Fraser et al. (1993) also noted that juvenile salmon were less aggressive at night and more tolerant of subordinates. However, this proximity may be due to the fact that an individual will defend a smaller area at night because the range at which prey can be detected decreases (Fraser and Metcalfe 1997) rather than to a total lack of territorial behaviour. Indeed, in a tributary of Lake Superior characterised by low winter flow, salmon were aggressively maintaining positions at temperatures as low as 3°C, with 53% of fish in dominance hierarchies (Healy and Lonzarich 2000). Despite the general consensus that there is a decrease in overt aggression between salmonids in winter, the results of this experiment indicate that competition for habitat and food does still occur.

The results suggest that aggregations and the general proximity of salmonids in winter, as reported by Cunjak and Power (1986) and Whalen and Parrish (1999), may result not only from a reduction in territorial aggression but from the limited availability of preferred habitat. Limited availability of winter habitats may play a pivotal role in the number of salmonids that a stream can support (Nickelson et al. 1992; Cunjak 1996; Whalen and Parrish 1999).

The greater tendency for fish to have been found in close proximity when in sympatry than when in allopatry is likely to be a result of the difference in the use of the water column by salmon and trout. Trout spent the majority of their time in the water column, whereas salmon were more likely to be on the substrate; this partitioning of the habitat may have allowed more fish to occupy the same area when there was a mixture of the two species. Bremset and Berg (1999) witnessed a similar segregation of salmon and trout, with trout holding position significantly farther from the substrate than salmon. The tendency for trout to spend a higher proportion of their time in the water column may be a result of several factors that are not mutually exclusive. Salmon have larger pectoral fins than trout and are thus better adapted for holding position on the substrate (Arnold et al. 1991). In addition, in artificial-stream environments, where most food is distributed in the water column rather than in the benthos, salmon always appear to move upwards to take food items at night. This is probably because the silhouette of a food item against the sky allows salmon to see and capture food items more easily. Thus salmon will sit on the bottom at night so they can get underneath all potential prey items (Valdimarsson and Metcalfe 1999). The positioning of trout above salmon in the water column when in sympatry will therefore deplete the food supply available to salmon. Thus, the shift to more diurnal activity by salmon may not be caused by overt aggression by trout, but by trout monopolising the available food in the water column. Salmon and trout may both spend less time on the substrate during the day to take advantage of surface drift, which is not visible to them during the night.

Implications

Recent experimental studies have shown that the trade-off between diurnal and nocturnal foraging is complex and may be related to a number of factors, such as food availability, feeding efficiency, life-history strategy, water velocity, and predation risk (Metcalfe et al. 1997, 1998, 1999). The results of this study suggest that interspecific competition can also affect the trade-off between nocturnal and diurnal foraging.

There are also widespread management implications arising from this study. First, the results reinforce previous reports of the importance of slow-flowing water to juvenile salmonids in winter. That this habitat requirement is often limited (Rimmer et al. 1985; Cunjak and Power 1986; Cunjak 1996) may have profound effects on those species known to depend upon it. The susceptibility of these species to habitat limitations may be further increased by the fact that, although during summer different age groups of fish select different habitats (Kennedy and Strange 1982), in winter young-of-the-year and post-young-of-the-year Atlantic salmon have been found to use similar microhabitats (Whalen and Parrish 1999). Secondly, the results indicate that competition between Atlantic salmon and brown trout for resources such as habitat and food is not restricted to the summer months, during which time it has previously been shown to occur (Kennedy and Strange 1986). The most immediate effect of this competition is that salmon mortality due to predation may increase during the winter if they are forced to become more active during the day (Metcalfe et al. 1999). Longer-term survival rates of salmon may also be affected by interspecific competition with trout. Salmon destined to smolt and migrate to the sea in the spring continue to grow during the winter months (Metcalfe et al. 1988). Since the survival of migrating individuals is size dependent (Lundqvist et al. 1994), reduced over-winter growth of salmon due to competition with trout may lead to decreased chance of survival.

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The potential of a stream to produce high numbers of good quality salmon smolts does not only depend on the availability of winter habitat, as suggested by Nickelson et al. (1992), but also on the presence of brown trout, which may affect the growth and survival of overwintering salmon. The importance of winter habitat to smolt production has recently been confirmed through a winter habitat modification study in which the over-winter survival and smolt production of coho salmon increased in treatment streams in comparison with unmodified control streams (Solazzi et al. 2000). Similar comparative studies are needed to determine the effects of brown trout on the over-winter survival and size of Atlantic salmon smolts.

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Analyses of length and age distributions using continuation-ratio logits

Anna Rindorf and Peter Lewy

Abstract: Sampling of length and age distributions of catches is important for the assessment of commercially fished stocks. This paper presents a new method for statistical analyses and comparisons of length and age distributions based on generalised linear models of continuation-ratio logits. The method allows statistical testing of the effects of both continuous and discrete variables. Further, by utilising the smoothness of length and age distributions as a function of length, the method provides more accurate estimates of these distributions than traditional methods. The observations are assumed to be multinomially distributed, but cases in which the variance exceeds that of this distribution may also be analysed. The implementation of the method in existing statistical analysis software is straightforward and is demonstrated using length and age distributions of the lesser sandeel, *Ammodytes marinus* Raitt.

Résumé : L'échantillonnage des distributions de fréquence des longueurs et des âges dans les captures est une étape importante dans l'évaluation des stocks des pêches commerciales. On trouvera ici une nouvelle méthode pour l'analyse statistique et la comparaison des distributions de fréquence des longueurs et des âges basée sur des modèles linéaires généralisés des logits cumulatifs supérieurs (continuation-ratio logits). La méthode permet d'éprouver les effets de variables tant continues que discontinues. De plus, en utilisant le lissage des distributions de fréquence des longueurs et des âges en fonction de la longueur, elle génère des estimations plus précises de ces distributions que ne le font les méthodes traditionnelles. On assume au départ que les distributions sont de type multinomial, mais on peut aussi analyser des cas où la variance dépasse celle de cette distribution. L'insertion de cette méthode dans les logiciels courants d'analyse statistique se fait sans problème, ce qui est illustré par l'utilisation des distributions de fréquence des longueurs et des âges du Lançon nordique, *Ammodytes marinus* Raitt.

[Traduit par la Rédaction]

Introduction

Length and age compositions of numerous commercially fished stocks are analysed on a regular basis to assess recruitment, stock biomass, and other aspects of the state of the stock. Samples for this purpose are obtained by either commercial-catch sampling, research surveys, or both. In a typical sampling programme, catch of a species is sampled by recording the total number or weight of the species caught, the length distribution of a subsample of the catch, and the age at length of a subsample of the length sample (Cotter 1998).

The multinomial distribution is frequently used to describe length and age distributions, and most authors compare these distributions by aid of the χ^2 test of homogeneity (Baird 1983; Zwanenburg and Smith 1983; Engås and Soldal 1992). However, the χ^2 distribution has the disadvantage of being an inaccurate approximation when the expected number of outcomes in a category is less than five (Cramér 1946), and the variance in the collected data is often greater than can be described by the multinomial distribution (Smith and Maguire 1983; Williams and Quinn 1998).

The calculation of catch in numbers at age from numbers at length is usually based on an age–length key. The traditional key is based on a two-way table of age and length in which the entries are number of fish at length and age (Fridriksson 1934). This key does not take the knowledge of fish growth into account. However, this relationship has been included in some investigations by assuming the length distribution of a particular age group to be normal (Schnute and Fournier 1980; Labonté 1983; Gudmundsdóttir et al. 1988).

This paper presents a new method to statistically analyse and compare samples of age and length distributions from different geographical areas, time periods, laboratories, etc. The object of the analyses is to enable the estimation of age and length distributions with the lowest possible uncertainty. One important step on the way is deciding whether or not to pool distributions from different strata. This multinomially based method provides a statistical tool for this decision that is comparable with the analysis of variance (ANOVA) in the case of normal distributed data.

The method is based on continuation-ratio logits as presented by Agresti (1990) and as previously applied by Kvist et al. (2000) to analyse age distributions. However, the method presented here has several additional advantages over previous applications. It can take the smoothness of length distributions into account even in cases where the length distribution is skewed or polymodal, and can be used in cases where the variance of the observations exceeds that

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of the multinomial distribution. The method utilises the knowledge of increasing mean fish age as a function of size without any assumptions being made regarding growth apart from the mean length of older fish being greater than that of younger. Further, it can easily be implemented in existing statistical-analysis software, and the effect of both categorical and continuous variables on the distribution can be tested. Methods to obtain the accuracy of estimated length or age distributions are provided as well. The method is demonstrated using survey data on length and age composition for the lesser sandeel, *Ammodytes marinus* Raitt.

Analysing ordinal multinomially distributed variables by continuation-ratio logits

Generalised linear models are a convenient tool for statistical analysis of variables following distributions in the exponential family (McCullaugh and Nelder 1989). Applying these to multinomial data enables model-selection, estimation, and testing techniques developed for generalised linear models to be employed. It is thus possible to simultaneously analyse several different length or age distributions. This implies that problems inherent in small samples can be reduced. Further, the ordinal nature of length and age measurements makes the application of the continuation-ratio logits, as presented by Agresti (1990), possible. This has several advantages when analysing length and age distributions, as shall be shown in the following.

Continuation-ratio logits can be used for any multinomial variable for which the outcomes are ordinally scaled. Assume that we have observations in ordinally scaled groups i and let p_i be the probability of the outcome falling in group i. The continuation-ratio logits are then defined as (Agresti 1990):

$$\log\left(\frac{p_i}{p_{i+1}+\ldots+p_n}\right), \quad i=1,\ldots,n-1$$

This logit corresponds to the binomial logit

$$\log\left(\frac{\pi_i}{1-\pi_i}\right)$$

where

$$\pi_i = \frac{p_i}{p_i+\ldots+p_n} = P(\text{index} = i \mid \text{index} \geq i)$$

for $i = 1,\ldots, n-1$. That is, π_i is the probability of the outcome being of index i conditional on the outcome being of index i or greater.

The continuation-ratio logits can be modelled separately or simultaneously in a generalised linear model of a binomially distributed variable, since the likelihood of a multinomial trial can be expressed as $\prod_{i=1}^{k-1} b[\pi_i, n_i, n_{i+}]$, where n_i is the number of outcomes in category i, n_{i+} is the number of outcomes in category i or greater, π_i is the conditional probability described above, and k is the number of outcome categories (Agresti 1990). Maximising the multinomial likelihood is thus equal to maximising the product of likelihoods of the conditional binomial distributions of each index. As shown by Agresti (1990), this implies that the continuation-ratio logit of each index may be modelled separately.

The continuation-ratio logits of the different outcomes are not independent observations. However, owing to the relationship between the multinomial likelihood and the likelihood of the conditional binomial distributions, maximising the multinomial likelihood is equivalent to maximising the product of the conditional binomial trials simultaneously. Thus, any software implementing maximum-likelihood techniques to analyse binomial data may be used to analyse ordinal multinomial data. This includes software designed for analyses of generalised linear models, such as SAS® and S-Plus® (McCullaugh and Nelder 1989; SAS Institute Inc. 1996; Mathsoft Inc. 1997). However, the original multinomial data must first be transformed into a data set containing the conditional binomial observations and the index of the outcome. For instance, if the multinomial data are as seen in the first two columns of Table 1, the data set to be analysed in the binomial model contains the index i, the number of outcomes in this category, n_i, and the number of outcomes of this order or greater, n_{i+} (Table 1, columns 1, 2, and 4).

If the data show greater variance than can be accounted for by the binomial variance, this may be incorporated in the generalised linear model by estimating a dispersion parameter (McCullaugh and Nelder 1989). The only constraint imposed by this method is that the dispersion parameter must be the same for all the observations included in the model, and thus for all conditional distributions, when multinomial data are analysed as described above.

Including the conditional probabilities of all indices simultaneously in one generalised linear model, it is possible to use the index as a continuous explanatory variable. The value of the conditional logit of group i can then be expressed by

$$\log\left(\frac{\pi_i}{1-\pi_i}\right) = \lambda(i)$$

where $\lambda(i)$ is a smooth function of i. The function $\lambda(i)$ can, for example, be modelled by a local nonparametric smoother or a polynomial in i. Alternatively, one model may be estimated for each ordinal group separately, if one does not wish to include the smooth relationship between the conditional probability and the index of the group. The effect of categorical factors on the conditional probability of group i may also be estimated by generalised linear models.

The model provides estimates of the conditional probabilities, $\hat{\pi}_i$, as well as the variances of these estimates. The estimated unconditional probabilities, $\hat{p}_i$, can be calculated from the conditional probabilities by applying the formula:

$$\hat{p}_1 = \hat{\pi}_1$$

$$\hat{p}_i = \hat{\pi}_i \cdot \left(1 - \sum_{j=1}^{i-1} \hat{p}_j\right) = \hat{\pi}_i \cdot \prod_{j=1}^{i-1} (1-\hat{\pi}_j), \quad i > 1$$

Approximations of the variance of $\hat{p}_i$ are easily obtained, if the variance of $\hat{\pi}_i$ has been estimated and if the conditional probabilities, $\hat{\pi}_i$, are modelled independently of each other. In this case, the estimates are uncorrelated and the variance

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Table 1. A multinomial data set with the ordinally scaled outcomes, i, and the corresponding data set for analysis in a generalised linear model of a binomially distributed variable with the explaining variable i and the observed conditional probabilities π_i.

Outcome (i)	No. of observations of i (n_i)	Observed probability of i (p_i)	No. of observations of index i or greater (n_{i+})	Conditional probability of i (π_i)
1	10	0.19	54	0.19
2	25	0.46	44	0.57
3	14	0.26	19	0.74
4	3	0.06	5	0.60
5	2	0.04	(2)[a]	(1)[a]

[a]As the conditional probability of the highest outcome is always one, the value in parentheses was not included in the analyses.

of these can be approximated by the first order Taylor approximation:

$$(1)\quad V(\hat{p}_1) = V(\hat{\pi}_1)$$

$$V(\hat{p}_i) \approx \hat{\pi}_i^2 \hat{A}_i^2 \sum_{j=1}^{i-1} \frac{V(\hat{\pi}_j)}{(1-\hat{\pi}_j)^2} + \hat{A}_i^2 V(\hat{\pi}_i), \quad i > 1$$

where

$$\hat{A}_i = \prod_{k=1}^{i-1} (1 - \hat{\pi}_k)$$

Correspondingly, the covariance between the unconditional probabilities, $\mathrm{COV}(\hat{p}_i, \hat{p}_j)$, can be calculated.

If the conditional probabilities of all groups are analysed simultaneously and one or more parameters are common to all groups, the predicted conditional probabilities, $\hat{\pi}_i$, are correlated. This complicates derivation of analytical approximations to the variance of $\hat{p}_i$. In this case, simulation studies provide a convenient method for estimating the variance.

A simulation procedure that can be used for this purpose is the parametric bootstrap (Davison and Hinkley 1997). In this type of analysis, observations are simulated from the parameter estimates obtained from the fitted model. The simulated observations are then used to re-estimate the parameters. This is done by repeating the entire procedure of model fitting on a number of simulated data sets. The parameter estimates obtained provide estimates of the simulated variance and mean of these parameters.

Estimation of the probabilities and their variance in the multinomial distribution

The estimated probabilities obtained by the traditional multinomial approach, $\tilde{p}_i$, are calculated as

$$\tilde{p}_i = \frac{n_i}{\sum_{j=1}^{k} n_j}$$

where n_i is the number of outcomes in category i and k is the number of outcome categories. Thus, this probability does not take the overdispersion potentially present in the data into account. The variance of the estimate is calculated as

$$(2)\quad V(\tilde{p}_i) = \frac{\tilde{p}_i(1-\tilde{p}_i)}{\sum_{j=1}^{k} n_j}$$

These estimates are referred to as the multinomial estimates in the following discussion.

Application of the method

Data analysed

The data used to demonstrate the method consist of catches of the lesser sandeel from three surveys conducted in the Firth of Forth east of Scotland in 1997 and 1998 (Fig. 1). Positions were initially chosen according to prior knowledge of sandeel distribution in the area and the same positions were dredged in both years. A modified scallop dredge was used each year to catch the sandeels while they were buried in the sediment (Winslade 1974). In 1998, the third survey was conducted using a van Ven grab to collect additional sandeels for ageing. This survey was performed at positions 2, 5, 7, and 8 (see Fig. 1*b*) within a week of the dredge survey.

At each station, an average of five dredge hauls of 15-min duration were carried out in both years. The total content of sandeels in all hauls was weighed separately. If time permitted, the entire content was counted and length measured to the nearest 0.5 cm below. Otherwise, a random subsample was counted, measured, and weighed.

In 1997, a random subsample of 10 sandeels was taken from each length group of the dredge catch at each position (provided that the catch exceeded 10 sandeels), and age determinations were made from otoliths of these fish at the Danish Institute of Fisheries Research. In 1998, the ages of only a small number of sandeels from the scallop-dredge catches were determined at the Danish Institute. To supplement these samples, sandeels collected in the grab survey were also used in calculating the age–length relationship. The ages of these samples were determined at the Institute of Fisheries Research Services, Marine Laboratory (Scotland, U.K.) and kindly supplied by S. Greenstreet.

Analysing length distribution of catch using continuation-ratio logits

The observations analysed in this model were distributions of the observed lengths grouped in 1-cm classes for each of the hauls taken. Thus, there were five length distributions at each position, on average. The number of fish in each length group was assumed to be multinomially distributed. The object of the model developed was to examine whether the length distribution of the catch varied from year to year and (or) from position to position. As these length

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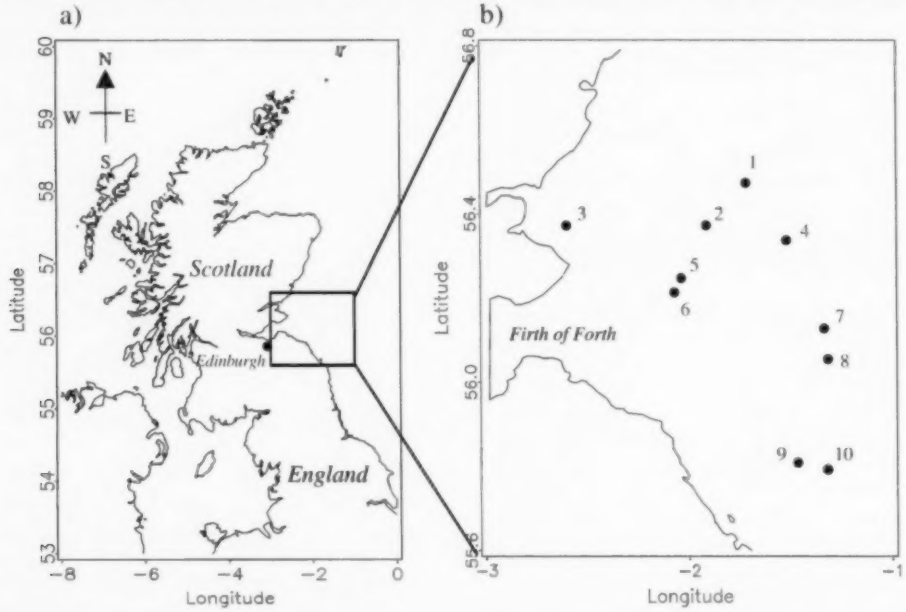
Fig. 1. (*a*) Map showing the location of the study area. (*b*) Enlargement of the inset in *a*, showing the locations of dredging positions 1–10.



categories are ordinal, the conditional probabilities can be modelled as described above.

Initially, the original multinomial data set was transformed into a data set containing the index of the outcome (length in this case), the number of outcomes in the category in trial *j*, the number of outcomes of this index or greater in trial *j*, and the additional variables year and dredging position. This is equivalent to the transformation illustrated in Table 1. If no fish of index *i* or greater were recorded in trial *j*, the conditional probabilities of index *i* and greater were missing in the trial. The length-dependent continuation-ratio logits were analysed in a generalised linear model of this binomial data set.

The minimum length observed was 7 cm and the maximum length for which conditional probabilities were calculated was 18 cm. The model of the continuation-ratio logits included a seventh degree polynomial in length and categorical parameters describing the effects of year and position:

$$(3) \qquad \log\left(\frac{\pi_{l,y,\text{pos}}}{1-\pi_{l,y,\text{pos}}}\right) = \lambda_{y,\text{pos}}(l) = \sum_{n=0}^{7} b_n(y,\text{pos})l^n$$

where

$$b_n(y,\text{pos}) = b_n + b_{n,y} + b_{n,\text{pos}} + b_{n,y,\text{pos}}$$

and *l* indicates length in group *l*, *y* indicates year, pos indicates position, and *b* indicates parameters to be estimated in the model. The seventh degree polynomial was chosen as the highest-order polynomial for which no convergence problems arose. These problems are due to lack of contrast in the data, which makes it difficult or impossible to find a global minimum of the deviance. The model was fitted using the SAS® GENMOD procedure (SAS® version 6.12 for Windows®; SAS® Institute 1996). The dispersion parameter was estimated by Pearson's X^2 statistic divided by the degrees of freedom. This parameter measures the deviation between the variance between samples (hauls) measured and the variance between samples expected in the multinomial distribution. A value of one indicates that the between-haul variation is completely described by random variation within five samples from a multinomial distribution. The model was reduced by eliminating insignificant factors from the model (*F* test, 5% level). Values of r^2 were calculated as the deviance explained by a particular factor divided by the total deviance.

The length proportions, $\hat{p}_{l,y,\text{pos}}$, estimated from the reduced generalised linear model (including the significant effects of year and position) were used to perform a parametric bootstrap analysis. The number of replicates was 1000, that is, 1000 independent multinomially distributed length composition data sets for all hauls were generated by simulation, using the estimated length distribution at the given year and position, as well as the total number of fish lengths measured in the particular haul, as input. For each of these 1000 equivalent data sets, a generalised linear model of the conditional probabilities was used to estimate the length proportions, $\hat{p}_{\text{sim},l,y,\text{pos}}$. The dispersion parameter in these models was fixed at one (no overdispersion). From the 1000 replicates, the mean, $\hat{p}_{l,y,\text{pos}}$, and the variance of the estimates were calculated.

The original data were slightly overdispersed, as data showed greater variance around the model than could be accounted for by the multinomial variance. The estimation of the length distributions from the original data included this problem by introducing a dispersion parameter (McCullaugh and Nelder 1989). However, overdispersion was not taken into account when simulating data or when building generalised linear models of the simulated data. Therefore the resulting variances estimated from the

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simulated data may be somewhat lower than the variances in the original data. However, the comparison between the variance calculated from the simulations and the variance in the original data does not affect the multinomial variance, as the dispersion parameter is assumed to be one in both cases.

The approximated analytically derived value of the variance of each length proportion, $\hat{p}_{l,y,pos}$, was calculated using eq. 1. These variances were compared with the simulated variances. Further, the estimated probabilities and the variances of these obtained by the traditional multinomial approach were calculated as well. These last two estimates do not take the variance between hauls (the overdispersion) into account.

Analysing age distributions for given length using continuation-ratio logits

As the number at age in a sample is also an ordinal multinomial variable, the model may equally well be applied to age distributions. In this case, the observations in the conditional binomial distribution are the number of fish of age a, given the age is a or greater. The relationship between the length of the fish and the continuation-ratio logit of the probability of being of a given age in a length group has been shown analytically by Kvist (1999) to be simple in certain cases (the results are summarised in the Appendix). Thus, if the length distribution of an age group follows a normal distribution, the relationship between length and the continuation-ratio logits can be approximated by a second-degree polynomial. A skewed length distribution such as a gamma distribution will lead to an approximately linear relationship between the continuation-ratio logits and length.

As the survey took place in early spring before the age-0 group settled in the area, only the age-1, -2, and -3+ groups were considered in the analyses. The primary aim of this model was to smooth the age distribution over lengths and not the age distribution at a given length. Therefore, to facilitate the interpretation of results, it was decided to model age groups separately and thus not smooth over multinomial orders as described in the section on length distributions.

As age determinations made by the two different laboratories may differ, and slight changes in age structure may have taken place in the 2 weeks between the surveys, a laboratory effect was included in the model. We assumed that this effect was independent of length, resulting in the full model:

$$\log\left(\frac{\pi_{a|l,y,pos}}{1-\pi_{a|l,y,pos}}\right) = c_a + c_{a,y} + c_{a,pos} + c_{a,y,pos} + d_{a,lab} + (g_a + g_{a,y} + g_{a,pos} + g_{a,y,pos})l + (h_a + h_{a,y} + h_{a,pos} + h_{a,y,pos})l^2$$

where a denotes age, l denotes length, $\pi_{a|l}$ denotes the conditional proportion of age a for given length l, lab denotes the laboratory at which the otoliths were read, and c, d, g, and h denote parameters to be estimated in the model.

Estimation of parameters in the model is performed by minimising the deviance. However, when length groups at either end of the length spectra with an observed probability of 0 or 1 were included, convergence problems occurred, owing to the inability of the minimisation routine (SAS® GENMOD (SAS® Institute 1996)) to detect a global minimum in deviance (McCullaugh and Nelder 1989). Therefore, these length groups were excluded from the data. This had virtually no effect on the estimated parameters and conditional probabilities, while eliminating the problems in the minimisation procedure. This meant that fish smaller than 8 cm or greater than 16 cm were excluded from calculations analyzing the probability of being age 1. Fish smaller than 11 cm were excluded from the analysis of the continuation-ratio logits of the age-2 group.

Estimation of the length distribution by age

The estimated length distribution for a given age group, $\hat{p}_{l|a}$, can be estimated by

$$(4) \qquad \hat{p}_{l|a} = \frac{\hat{p}_l \hat{p}_{a|l}}{\sum_l \hat{p}_l \hat{p}_{a|l}}$$

where $\hat{p}_{a|l}$ denotes the estimated probability of being a years old for a given length l, and $\hat{p}_l$ denotes the estimated probability of being length l. As the length distribution may differ with year and position, a length distribution was estimated for each of the year–position combinations for which more than 100 sandeels were measured. The average length distribution over positions of an age group in a given year was calculated as a weighted average, using the mean number caught at each position as weights.

Results

Length distributions

Observed length distributions, estimated multinomial length distributions, and 95% confidence limits of the estimates for two of the dredging positions are displayed in Fig. 2. The results of testing the significance of the parameters in the full model of length distribution (eq. 3) show that the parameters $b_n(y,pos)$ for $n = 6$ and $n = 7$ were not significantly different from zero, which means that the continuation-ratio logit could be described by a fifth degree polynomial in length (Table 2). The fifth degree parameter, b_5, does not vary with year and position. The part of the polynomial that is fourth degree or less varies with year, position, or their crossed effect, indicating that the length distribution is not simply changed towards larger or smaller fish at all positions from 1 year to the next. Note that the polynomial parameters estimated are not of particular interest, but are merely tools used to model the fact that length distributions are usually smooth functions of length. The predicted length distributions for different positions are correlated and, therefore, there is a gain in the precision of the predictions from incorporating this into the model as opposed to examining each position separately. The proportion of the total deviance explained by the model is high (93%). The rather high number of parameters estimated (91) should be compared with the multinomial alternative of estimating 271 probabilities (one for each length group at each year and position). The deviation between positions is greater than the deviation between years (15% as opposed to 8.8% of total deviance). It is interesting to note that the greater part of the variation between

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Fig. 2. Length distributions at dredging positions 1 (*a*) and 6 (*b*) in 1998; + signs indicate the observed probability, the solid line indicates the probability estimated by the multinomial distribution, and the hatched lines indicate the 95% confidence limits of the estimate (calculated using eq. 2).

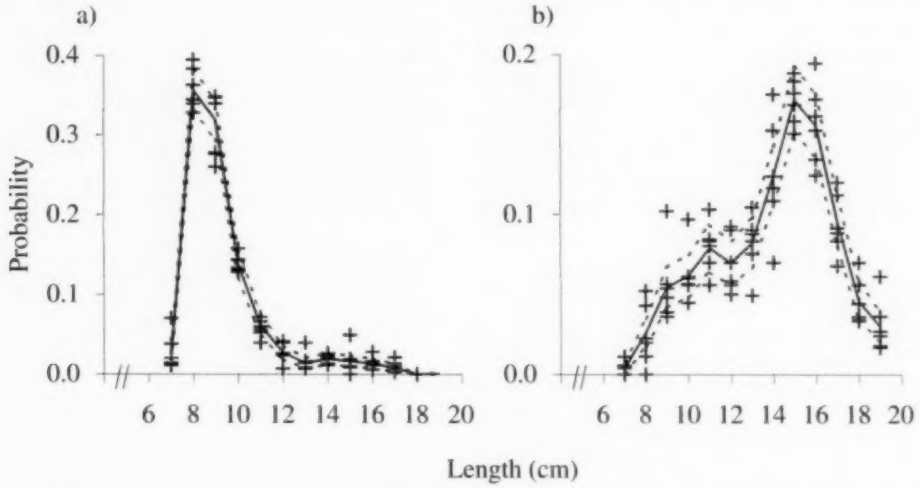

Table 2. Generalised linear model of the continuation-ratio logit of the probability of being of length *l*.

Source	Deviance	df	*F* (Type 1)	*P* > *F*	r^2	Cumulative r^2
Total	24 045	1036				
$f_1(l)$	13 901	5	1585	0.0001	0.578	0.578
$f_2(l,\text{year})$	2 120	4	292	0.0001	0.088	0.666
$f_3(l,\text{position})$	3 616	36	55	0.0001	0.150	0.817
$f_4(l,\text{year,position})$	2 654	36	41	0.0001	0.110	0.927
$b_{4,\text{pos}}l^4$	74	9	4.7	0.0001	0.003	0.930
$b_{4,\text{y}}l^4$	1	1	0.8	0.3594	0.000	0.930
$b_{4,\text{y,pos}}l^4$	13	9	0.8	0.6290	0.001	0.931
$b_{5,\text{y}}l^5$	1	1	0.8	0.3608	0.000	0.931
$b_{5,\text{pos}}l^5$	36	9	2.3	0.0170[a]	0.001	0.932
$b_{5,\text{y,pos}}l^5$	9	9	0.6	0.8310	0.000	0.933

Note: $f_1(l) = \sum_{n=0}^{5} b_n l^n$, $f_2(l,\text{year}) = \sum_{n=0}^{3} b_{n,\text{y}} l^n$, $f_3(l,\text{position}) = \sum_{n=0}^{3} b_{n,\text{pos}} l^n$, and $f_4(l,\text{year,position}) = \sum_{n=0}^{3} b_{n,\text{y,pos}} l^n$. See text for definition of b_n.

[a]The parameters no longer have a significant effect when the model is reduced.

positions can be explained by a greater proportion of smaller or larger fish at some positions in both years, rather than by a general trend towards larger fish in one of the years. The data were slightly overdispersed (dispersion parameter = 1.45).

The estimated conditional probabilities, $\hat{\pi}_{l,\text{y,pos}}$, the corresponding observed values, and the 95% confidence limits of the estimates are plotted against length in Fig. 3 for the two dredging positions for which the multinomial length distribution is seen in Fig. 2. Correspondingly, the unconditional length distributions predicted by the model for the same two positions are shown in Fig. 4, along with the simulated confidence limits of this prediction. The confidence limits obtained by the suggested model are clearly smaller than the confidence limits of the multinomial probability usually employed (Fig. 2).

Comparison of simulated and approximated variance

The length distributions estimated by applying the model to the simulated data did not differ significantly from the input length distributions from which the simulations were derived (the probability of difference in no case exceeded 0.50), indicating that the method provides unbiased estimates of the length proportions. The relative difference between the standard deviation of the predicted length distribution approximated by eq. 1 and the standard deviation calculated from the simulations is shown in Fig. 5*a*. If the covariances assumed in eq. 1 to be zero are in fact negative, then the approximated standard deviation will be an overestimate compared with the true value for all groups but the smallest. The relative difference between the variances is below 0.5 for the 7 and 8 cm length groups, but increases rapidly with length, indicating substantial negative correlation between the estimated conditional probabilities.

Comparing the simulated standard deviation of the model predictions with the standard deviation of the unsmoothed multinomial probabilities (Fig. 5*b*), the traditional method

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Fig. 3. Probability of being a given length conditional on being at least this length, $\hat{\pi}_{l,y,pos}$, at dredging positions 1 (*a*) and 6 (*b*) in 1998; the + signs indicate the observed probability, the solid line indicates the probability predicted by the model, and the hatched lines indicate the 95% confidence limits of the predicted mean.

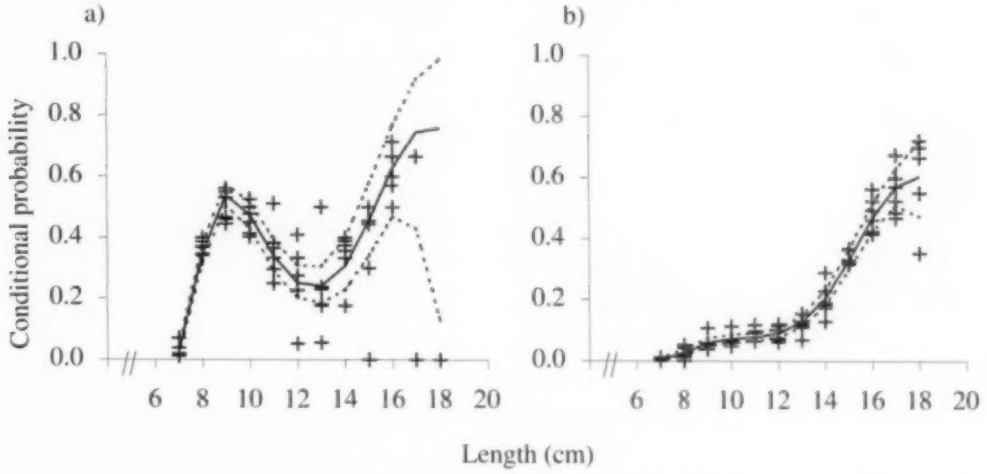
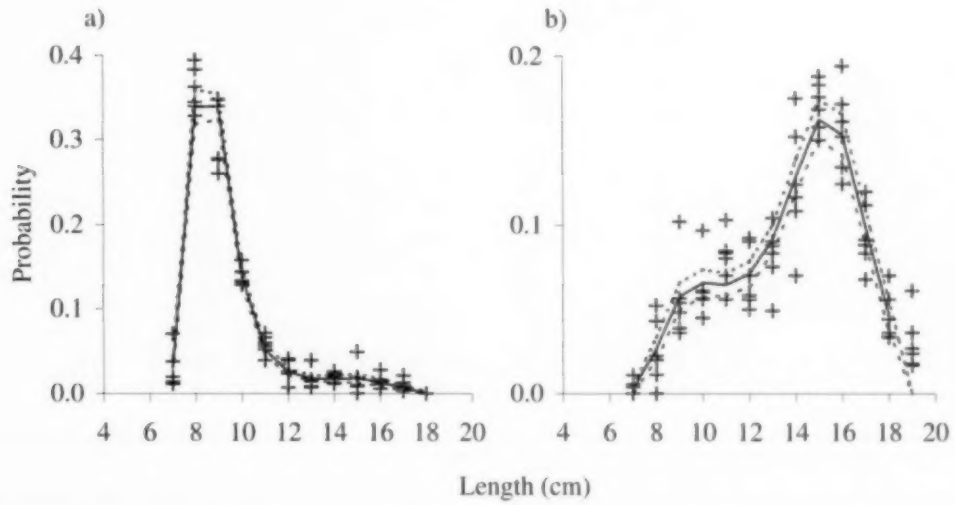


Fig. 4. Length distribution at dredging positions 1 (*a*) and 6 (*b*) in 1998; the + signs indicate the observed probability of being length *l*; the solid line indicates the probability predicted by the model, and the hatched lines indicate the 95% confidence limits of the mean (simulated).



consistently provides estimates with higher standard deviation. The reduction in standard deviation ranges from a minimum of 22%, on average, for the smallest length group to a maximum of 54%, on average, for the 14 cm length group, indicating the advantage of utilising the knowledge of the smoothness of the catch as a function of length.

Age distribution for given length

Leaving out insignificant effects, the model of the conditional probability of being age 1 became

$$\log\left(\frac{\pi_{1l,y,pos}}{1-\pi_{1l,y,pos}}\right) = c_1 + c_{1,y} + c_{1,pos} + g_1 l$$

Thus there was no significant effect of length squared or laboratory. The reduced model describes the data well, explaining 91% of the total deviation (Table 3; Fig. 6). In each length group, the number of sandeels for which age was determined varies, with a mean of 16 for the 8–12 cm length groups and a mean of three for the 13–16 cm length groups. The observed value of $p = 1$ in Fig. 6*a* at 14 cm is derived on the basis of one fish for which age was determined. Further, the logits of 0 and 1 are not defined, and these can therefore not be plotted in Fig. 6*b*. However, these values are included in the model-fitting process and thus the slope of the line appears somewhat high.

By far the most important factor influencing the conditional probability is length (67%), demonstrating the importance of including this information in the model. The year

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Fig. 5. Relative differences in standard deviation by length (indicated by + signs), calculated as $(std_{estimated} - std_{simulated}) / std_{simulated}$; the solid line indicates the average relative difference. (*a*) The value of $std_{estimated}$ calculated by assuming covariances to be zero (eq. 1). (*b*) The value of $std_{estimated}$ calculated by the multinomial distribution (eq. 2).

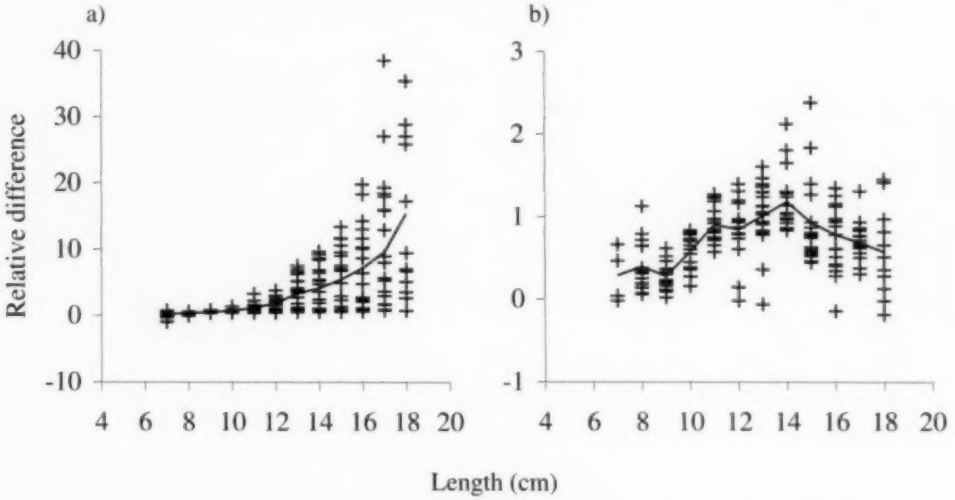

Table 3. Model of probability of being age 1.

Source	Deviance	df	*F* (Type 1)	*P* > *F*	r^2	Cumulative r^2
Total	2791	403		0.0001	0.671	0.671
$g_1 l$	1872	1	2225	0.0001	0.197	0.867
$c_{1,y}$	549	1	653	0.0001	0.039	0.906
$c_{1,pos}$	108	9	14	0.0059[a]	0.003	0.909
$d_{1,lab}$	7	1	7.7	0.0027[a]	0.007	0.915
$c_{1,y,pos}$	19	7	3.2	0.0660	0.005	0.920
$g_{1,y} l$	13	9	1.8	0.3215	0.000	0.921
$h_1 l^2$	1	1	1.0	0.0356[a]	0.004	0.925
$h_{1,y} l^2$	12	6	2.3	0.2133	0.000	0.925
$g_{1,pos} l$	1	1	1.6	0.2666	0.000	0.926
$g_{1,y,pos} l$	1	1	1.2	0.8213	0.001	0.927
$h_{1,pos} l^2$	4	9	0.6	0.9878	0.000	0.927
$h_{1,y,pos} l^2$	1	6	0.2			
Residual	203	335				

[a]The parameters no longer have a significant effect when the model is reduced.

effect is the second most important factor (20%). Therefore, the same age–length key should not be applied in the 2 years. The lack of evidence of a second-degree effect of length indicates that the length distribution by age is either skewed (resembling a gamma distribution) or that the variances of the length distributions of the age-1 and age-2 groups are similar (Appendix). The percentage reduction in standard deviation obtained by using the model ranges from 35% (at 12 cm) to 71% (at 14 cm).

For the age-2 group, length once again explains more than half the variation in the probability ($r^2 = 0.517$, $P < 0.0001$). As for the age-1 group, year effect is the second most important parameter, with an r^2 of 0.139. In contrast with the model of the conditional probability of being age 1, the reduced model showed significant laboratory effects ($r^2 = 0.018$, $P < 0.0002$), perhaps owing to the increased difficulty in determining the age of older fish. When a significant crossed effect between year and length is included as well ($r^2 = 0.010$, $P < 0.0068$), the proportion of the total deviation explained is somewhat less than in the model of age 1.

Length distribution by age

The length distributions estimated by eq. 4 for sandeels in the age-1 and age-2 groups in 1997 and 1998 are unimodal and smooth, and the distribution for the 2 year olds is slightly broader than the distribution for the 1 year olds (Fig. 7). The distributions of the age-1 group in both years and of the age-2 group in 1997 are symmetrical and may be approximated by normal distributions. The distributions of the age-2 group in 1998 and the age-3+ groups in both years are very skewed, but the distributions of the age-2 and age-3+ groups in 1998 also show a tendency to bimodality.

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Fig. 6. Probability of being age 1 (*a*) and the logit of this probability (*b*) as a function of length at dredging position 5 in 1997. The + signs indicate the observed values (calculated by haul), the solid line indicates the values predicted from the model, and the hatched lines indicate the 95% confidence limits of the predicted values.

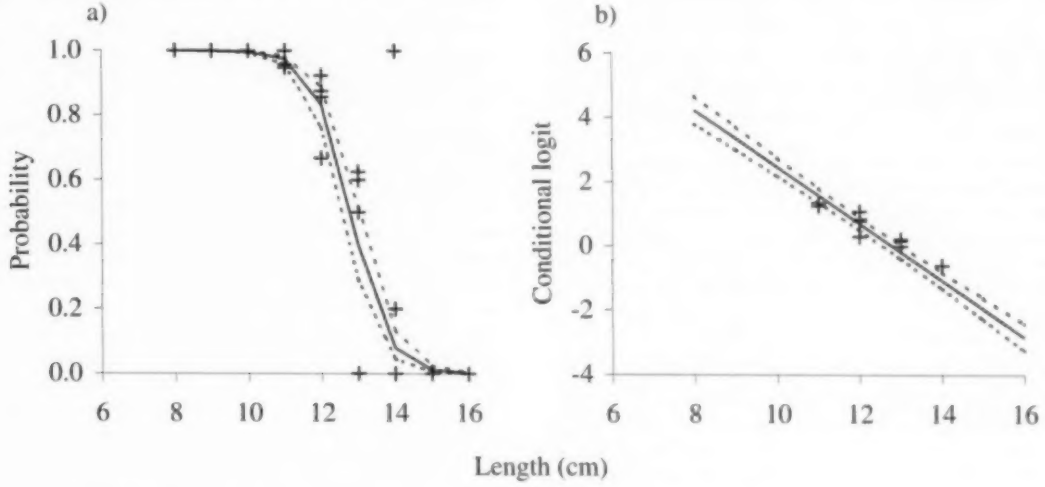
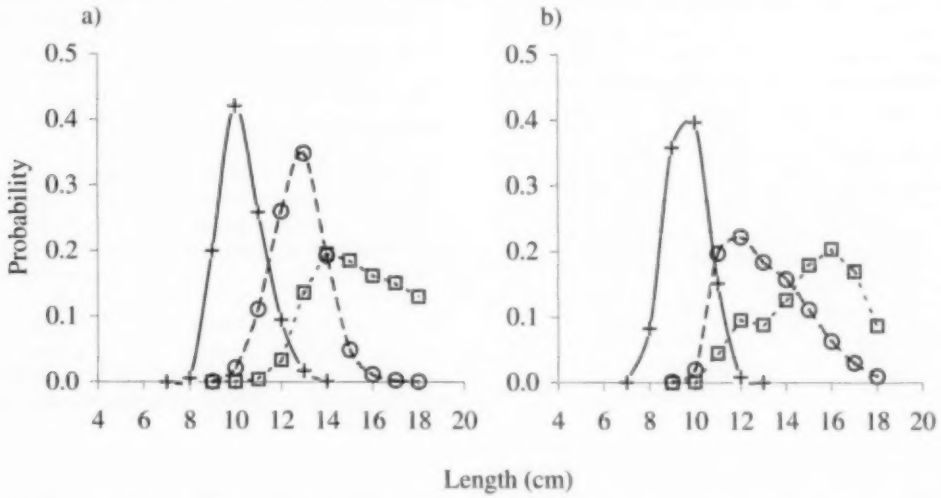


Fig. 7. Estimated length distribution of age-1 (+), age-2 (○), and age-3+ (□) sandeels in (*a*) 1997 and (*b*) 1998.



Whether this is caused by the actual length distribution or by sampling problems, such as problems with the accuracy of age determinations in this year, is not possible to determine from this study.

Discussion

The method suggested here has three main advantages compared with the standard use of the multinomial distribution. One is that entire multinomial distributions, such as length distributions or age distributions by length, may be modelled using generalised linear models in existing software. The theory of model selection, testing of hypotheses, estimation, and prediction developed for these models may be used directly. Further, it is likely that the χ^2 distribution is a better approximation of the distribution of the deviance than the usual test in the multinomial distribution and that the power of the test of the new method is greater than the multinomial χ^2 test, as the new method utilises the ordinal nature of the outcomes by simultaneously utilising data for all length groups contrary to the multinomial approach. However, the actual improvement in the example shown here has not been tested. Secondly, including the smoothness of the length distribution and the age distribution as a function of length is straightforward and improves the precision of the estimates. The suggested method therefore provides more accurate estimates of length and age distributions than the multinomial approach. Furthermore, estimates are unbiased. Thirdly, overdispersion is easily incorporated in the analyses, allowing the data to vary more than expected in a traditional multinomial distribution. If the variance in the collected data is greater than expected in the multinomial

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case, this may be addressed alternatively by analysing compound multinomial distributions in which the probabilities of the different outcomes are assumed to vary between samples (Smith and Maguire 1983) or by assuming the probabilities of the outcomes to be dirichlet distributed (Williams and Quinn 1998). However, neither of these methods incorporates the smoothness of the distributions, and they are both sensitive to the inadequacy of the χ^2 distribution in the small-sample case.

We have not been able to find other examples in which generalised linear models and continuation-ratio logit analyses have been used simultaneously for all outcomes of a multinomial distribution thus enabling the relationship between the index of the group and the probability to be modelled as smooth. However, separate fitting for each index group as suggested by Agresti (1990) has been used in analyses of age distributions (Kvist et al. 2000).

The results of a model of the continuation-ratio logits are often more easily interpreted than a model of the corresponding multinomial distribution, as a positive factor in the continuation-ratio logit model may simply be interpreted as, for example, "increasing the probability of young fish," in the case of analyses of age distributions. This was demonstrated in the reduced model of the conditional probability of being age 2, where one laboratory consistently placed a higher proportion of the fish in the age-2 group than the other laboratory. An additional advantage of the present approach compared with the traditional multinomial method is the ability to predict or interpolate length distributions or age compositions by length as well as the associated variances. This is particularly useful in cases where, in a particular intermediate-length group, either no fish were caught, all fish were of a particular age, or no ages were determined for the fish. Traditional methods are unable to estimate the proportion of a given age at this length. These problems may be approached alternatively by assuming the length distribution of the age groups to be normal (Labonté 1983; Gudmundsdóttir et al. 1988) or some other known distribution (Martin and Cook 1990). However, the method presented in this paper has the advantage of being able to describe unknown skewed or bimodal as well as normal length distributions by fitting the best possible polynomial in length to the logit, thereby letting the data dictate the age-specific length distribution. When estimates from the models of length and age distributions are combined, they provide smooth length distributions of the age groups. The data are not smoothed to the extent that problems in the input data become invisible, as is the case when length distributions at age are assumed to be normal. Further, length at age in the catch sample is sometimes known to deviate from normality, for example, if catchability increases with length. The youngest age groups in a trawl-survey catch will then have a skewed length distribution. Catchability problems, ageing problems, bimodal size at age distributions, etc., are still detected when applying the method. In the simple case in which length distributions of fish of age a and age $a + 2$ do not overlap, the relationship between the length distribution of the age group and the continuation ratio logit model is simple, as shown analytically by Kvist (1999) and summarised in the Appendix. In the data analysed here, the overlap between the age-1 and age-3+ groups is indeed limited, and so the approximation is reasonable. However, slower-growing species or species with large variations in growth rate may exhibit overlap between the length distributions of several age groups. In this case, the validity of the approximation of the continuation-ratio logit to a second-degree polynomial in length remains to be examined. Nevertheless, even if the approximation is not valid, a polynomial in length may still provide the best practical description of the continuation-ratio logit as a function of length.

When separate models of the continuation-ratio logit of the proportion at age by length are fitted, analytical calculation of the variance of the untransformed age proportions is simple. However, for length distributions in which the length-dependent logits have been analysed simultaneously, it is only possible to obtain poor approximations of the variance of the untransformed length proportions. The approximations may be appropriate for a small number of length groups, but Monte Carlo methods are needed to obtain reliable variance estimates for all length groups. Furthermore, overdispersion should be taken into account when performing the simulations, if the dispersion parameter differs from one. Thus, comparing mean lengths in one or more broader length groups by the χ^2 test of homogeneity (Baird 1983; Zwanenburg and Smith 1983; Engås and Soldal 1992) may, in many cases, provide adequate estimates for which variances are more easily calculated.

The method presented here provides estimates of age composition in a sample with a higher precision than the traditional methods. The model further provides opportunities to test for the effect of factors such as time or area, which will enable the analyst to decide when to join length distributions or age–length keys and when to apply them separately, to obtain the greatest accuracy in the results. In the past, extensive research has been devoted to obtaining age–length keys with the highest precision at the lowest possible cost (Kimura 1977; Baird 1983; Lai 1993). Analysing data in the way suggested here may alter the optimal sampling strategy and may even allow the same precision to be obtained with fewer samples and thus lower cost. The implication of this for the optimal sampling strategy remains to be determined.

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Appendix

The relationship between the length and the continuation-ratio logit of the probability of being a particular age in the catch given the length can analytically be shown to be simple (Kvist 1999). A brief summary of the derivation of the relationship is given in the following.

The probability of a fish in the sample being age a, given the fish is of length l, $p_{a|l}$, can be expressed by

$$p_{a|l} = \frac{p_{l|a}p_a}{\sum_a p_{l|a}p_a}$$

where $p_{l|a}$ is the probability of a fish in the catch being length l given the age of the fish is a and p_a is the probability of a fish in the catch being age a. Note that this relationship is not affected by stratifying age sampling by length, as long as age samples are taken at random within each length group. The probability, $p_{a|l}$, is thus proportional to the length distribution at age a and the probability of being at age a.

The continuation-ratio logit for $p_{a|l}$ is then

$$\text{(A1)} \quad \log\left(\frac{p_{a|l}}{\sum_{i\geq a+1} p_{i|l}}\right) = \log\left(\frac{p_{l|a}p_a}{\sum_{i\geq a+1} p_{l|i}p_i}\right)$$

If the length distribution at age, $p_{l|a}$, can be described by a normal distribution, eq. A1 can be written as

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$$\log\left(\frac{p_{al}}{\sum_{i\geq a+1} p_{il}}\right) = \log p_a - \log\sigma_a - \frac{(l-\mu_a)^2}{2\sigma_a^2} - \log\left(\sum_{i\geq a+1}\frac{p_a}{\sigma_i}\exp\left(-\frac{(l-\mu_i)^2}{2\sigma_i^2}\right)\right)$$

where μ_a and σ_a are mean and standard deviation, respectively, of the length distribution of age group a.

This is unfortunately not a simple function of l. However, if age group a can be assumed to overlap only with age group $a + 1$ and not with age group $a + 2$, the expression may be approximated by

$$\log\left(\frac{p_{al}}{\sum_{i\geq a+1} p_{il}}\right) = \log p_a - \log\sigma_a - \frac{(l-\mu_a)^2}{2\sigma_a^2} - \log p_{a+1} + \log\sigma_{a+1} + \frac{(l-\mu_{a+1})^2}{2\sigma_{a+1}^2}$$

Thus, the continuation-ratio logit may be approximated by a second-degree polynomial in length, provided age group a does not overlap with age group $a + 2$. Furthermore, if the standard deviations of age group a and age group $a + 2$ are equal, the continuation-ratio logit may be expressed by a first-degree polynomial in length.

The approximation is equally simple if the length distribution of the age groups is assumed to be gamma distributed, with mean $k_a\beta_a$ and variance $k_a\beta_a^2$, as the expression then becomes

$$\log\left(\frac{p_{al}}{\sum_{i\geq a+1} p_{il}}\right) = \log p_a - \log\Gamma(k_a) - k_a\log\beta_a + (k_a - 1)\log l - \frac{l}{\beta_a} - \log p_{a+1} + \log\Gamma(k_{a+1}) + k_{a+1}\log\beta_{a+1}$$

$$- (k_{a+1} - 1)\log l + \frac{l}{\beta_{a+1}}$$

where

$$\Gamma(k) = \int_0^\infty t^{k-1}\exp(-t)\mathrm{d}t$$

and overlap between age groups a and $a + 2$ is still assumed to zero. This expression is linear in $\log l$ and is approximately linear in l in the range considered in the present paper.

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Fishing on ecosystems: the interplay of fishing and predation in Newfoundland–Labrador

Alida Bundy

Abstract: In the early 1990s, Atlantic cod, a major component of the Newfoundland–Labrador ecosystem, suffered a stock collapse, and other groundfish stocks such as American plaice and yellowtail flounder seriously declined. This paper explores whether the relative effects of predation and fishing alone can account for these ecosystem changes. The Newfoundland–Labrador ecosystem was first modelled with a mass balance model for a time period in the mid-1980s when the groundfish biomass was relatively stable. This provided the starting point for simulations using a trophodynamic simulation model, Ecosim. A series of simulations were run, under different assumptions about energy control, to address the larger question "can the effects of fishing and predation account for the changes observed in the ecosystem?" The collapse and nonrecovery of cod was replicated, assuming top-down energy control. Other control assumptions were less successful. While groundfish stocks collapsed, seal populations and invertebrates such as shrimp and snow crab increased in abundance. The model predicted these increases, while a simulated increase in harp seals further repressed the recovery rate of cod. It was concluded that these results are consistent with the hypothesis that the collapse of cod was caused by excess fishing and that cod recovery is retarded by harp seals.

Résumé : Au début des années 1990, la Morue franche, une des composantes principales de l'écosystème Terre-Neuve–Labrador, a subi un effondrement de stock, et les stocks d'autres poissons de fond, tels que la Plie canadienne et la Limande à queue jaune, ont connu d'importants déclins. On examine ici si les effets relatifs de la prédation et de la pêche peuvent, à eux seuls, expliquer ces modifications de l'écosystème. L'écosystème Terre-Neuve–Labrador a d'abord été modélisé à l'aide d'un bilan massique sur une période du milieu des années 1980 au moment où la biomasse des poissons de fond était assez stable. Cela a fourni un point de départ pour des simulations faites à l'aide d'Ecosim, un modèle de simulations tropho-dynamiques. Plusieurs simulations ont été faites correspondant à des hypothèses diverses sur le contrôle de l'énergie, dans le but de répondre à la question générale « est-ce que les effets de la pêche et de la prédation peuvent expliquer les changements survenus dans l'écosystème? » L'effondrement et l'absence de récupération du stock de morues ont pu être simulés, lorsqu'on supposait un contrôle descendant de l'énergie; si on posait en hypothèse d'autres types de contrôle, les résultats laissaient à désirer. Alors que les stocks de poissons de fond s'effondraient, les populations de phoques et d'invertébrés, tels que les crevettes et le Crabe des neiges, augmentaient de densité. Le modèle a prédit ces accroissements; lorsqu'on simulait un accroissement supplémentaire de la population du Phoque du Groenland, le taux de récupération de la morue déclinait encore plus. En conclusion, ces résultats s'accordent avec l'hypothèse qui veut que l'effondrement du stock de morue soit dû à une surpêche et que son rétablissement soit inhibé par les Phoques du Groenland.

[Traduit par la Rédaction]

Introduction

Fisheries on the East Coast of Canada suffered catastrophic collapses of their groundfish stocks in the early 1990s. Moratoria have been in place for Atlantic cod since 1992, for American plaice since 1995, with no directed fishery in 1994, and for yellowtail flounder from 1995 to 1997, with no directed fishery in 1994. The social and economic consequences have been disastrous for fishing communities, while the full extent of the ecological consequences is still to be realised. Since the collapse of the Northern cod in 1992, various theories have been proposed for its demise. These include increased natural mortality due to environmental factors (deYoung and Rose 1993; Lear and Parsons 1993), increased natural mortality due to an increasing harp seal population (Fisheries Resource Conservation Council, press release, May 24, 2000), overfishing (Hutchings and Myers 1994; Myers et al. 1996), and inaccuracy in biomass estimation (Walters and Maguire 1996).

It is generally agreed that these factors may have all contributed to the collapse of the groundfish stocks. A current question of greater concern is what is preventing most of these stocks from recovering. In 2000, the moratoria are still in place for cod and American plaice, with no indication that recovery is occurring. The fishery for yellowtail flounder reopened in August 1998 following a clear increase in biomass and expansion of stock range (Walsh et al. 2000).

The roles of fishing and predation in both the collapses of these stocks on the Newfoundland–Labrador Shelf and the prolonged lack of recovery of cod and American plaice are explored here using a trophodynamic simulation model, Ecosim (Walters et al. 1997). The aim of the research was to

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simulate the fishing effort and mortality changes that occurred in the Newfoundland–Labrador fisheries between 1985 and the present and to determine whether the ecological changes that have been observed in these fisheries can be explained by the effects of fishing mortality and trophic interactions. More specific research questions asked here were can the collapse and lack of recovery of the cod and American plaice be replicated using a mass balance and trophodynamic approach, can other ecosystem changes such as apparent increasing shrimp and crab populations be simulated, and do seals have a negative effect on the recovery of cod?

The Newfoundland–Labrador fishery was modelled for the period 1985–1987 in North Atlantic Fisheries Organisation (NAFO) Div. 2J3KLNO to a depth of 1000 m (Bundy et al. 2000) using the mass balance model Ecopath (Christensen and Pauly 1992; ICLARM 1995). This model was severely constrained by biomass estimates for the major species in the system and is thus considered representative of the ecosystem for that time period. An analysis of the relative roles of fishing and predation, in this steady-state representation, indicated that harp seal predation and cod predation accounted for a greater part of the total mortality on all fish groups than the fishery. However, for mature fish of species such as cod, American plaice, and Greenland halibut, the effects of fishing mortality were much greater than predation mortality. These results indicate that fishing mortality may be a major determinant in the population dynamics of some of these commercial species. However, there was also significant "other" mortality for American plaice that could be due to discarding, disease, starvation, temperature, or environmental changes.

These Ecopath mass balance results are used as input data for the trophodynamic simulation model Ecosim. Ecosim is used to simulate forward in time, under different scenarios of fishing mortality and different assumptions of energy flow control, in an attempt to gain an ecosystem perspective of the groundfish collapses and lack of recovery in the Newfoundland–Labrador fishery.

In order to recreate the patterns of fishing mortality that have occurred in the fishery since the mid-1980s, simulations were conducted under five scenarios of changes in fishing mortality (Fig. 1). These scenarios are intended as approximations of actual fishing mortality, and for simplicity, moratoria were presumed to begin in 1994. Each scenario includes the changes of the preceding scenario, that is, scenario 2 includes scenario 1, scenario 3 includes scenarios 2 and 1, and so on. In this way, it may be possible to judge the relative effects of the fishing mortality changes.

Different assumptions of energy control are modelled, as two competing theories of energy flow control dominate the ecological literature (Matson and Hunter 1992): top-down control with trophic cascades (Carpenter et al. 1985; Carpenter and Kitchell 1993) versus bottom-up, donor control (Hall et al. 1970; Hunter and Price 1992). However, the notions of bottom-up and top-down control are not well defined. Operationally, a bottom-up regime is defined by a response to changes in productivity at the lowest trophic levels. An increase in productivity at the bottom of the trophic system leads to an increase in the productivity and abundance at all higher trophic levels. Each trophic level is food limited (Power 1992), not predator limited. Thus, an increase in the abundance of a predator does not lead to an increase in the mortality rate on the prey. Predation is proportional to the biomass of the prey, flow is controlled by prey abundance, and the mortality rate is stable over time.

The operational definition of top-down control is that an increase in the abundance of a top predator leads to the prediction of a trophic cascade. Here, flow is predator controlled. An increase in productivity at the bottom would lead only to an increase in biomass at the top of the food chain. The biomass of lower levels would not increase because the mortality rate incurred by them would increase due to an increase in the abundance their predators. Only their productivity would increase.

Methods

The trophodynamic simulation model Ecosim reexpresses the linear equations of Ecopath as difference and differential equations that dynamically respond to changes in fishing mortality and biomass based on (Walters et al. 1997)

$$(1)\qquad \frac{dB_i}{dt} = g_i \sum_{j=1}^{n} c_{ji}(B_i, B_j) - M_i B_i - F_i B_i - \sum_{j=1}^{n} c_{ij}(B_i, B_j)$$

where dB_i/dt is the change in biomass or growth rate of group i during time interval dt, g_i is the gross efficiency of group i, $c_{ij}(B_i, B_j)$ is a function used to predict consumption from the biomass of the prey i and the predators j, M_i is nonpredation or "other" mortality, and F_i is fishing mortality of group i. It is a deterministic model that provides quantitative output, although results should be interpreted in terms of direction and magnitude of change. At present, there is no formal routine for taking uncertainty into account, although uncertainty in the mass balance model can be explored in Ecopath.

Ecosim enables the user to split groups into two size-classes to allow for trophic ontogeny. These groups are tracked in terms of both biomass and numbers. Additional data are required for the split groups, the age and length of maturity and the von Bertalanffy growth parameter, K.

Bottom-up control and top-down control are modelled in Ecosim based on the foraging arena concept by assuming that prey are not vulnerable to predation at all times. Prey are split into vulnerable and invulnerable components and there is an exchange rate between them. Top-down control is simulated by making the exchange rate high, meaning that a large part (or all) of the prey biomass is vulnerable to predation. Bottom-up control is simulated by making the exchange rate very low. This limits the amount of prey that are vulnerable to predation (for example, by hiding in crevices or between rocks):

$$(2)\qquad c_{ij}(B_i, B_j) = \frac{a_{ij} v_{ij} B_i B_j}{2v_{ij} + a_{ij} B_j}$$

where, a_{ij} is the instantaneous mortality on the prey B_i caused by one unit of predator biomass B_j and v_{ij} is the maximum instantaneous mortality rate that B_j can exert on B_i or the parameter that represents the rate of exchange between invulnerable and vulnerable states. For further details, see Walters et al. (1997).

The Newfoundland–Labrador Ecopath model has 31 groups: three marine mammal groups, one seabird group, 17 fish groups, nine invertebrate groups, and one detritus group (see Appendix). Some groups such as the flounders or the large demersal feeders are aggregated groups, where the species are aggregated on the basis of similarity of size and ecological role. The flounders, for example, comprise yellowtail, witch, and winter flounder (Table 1).

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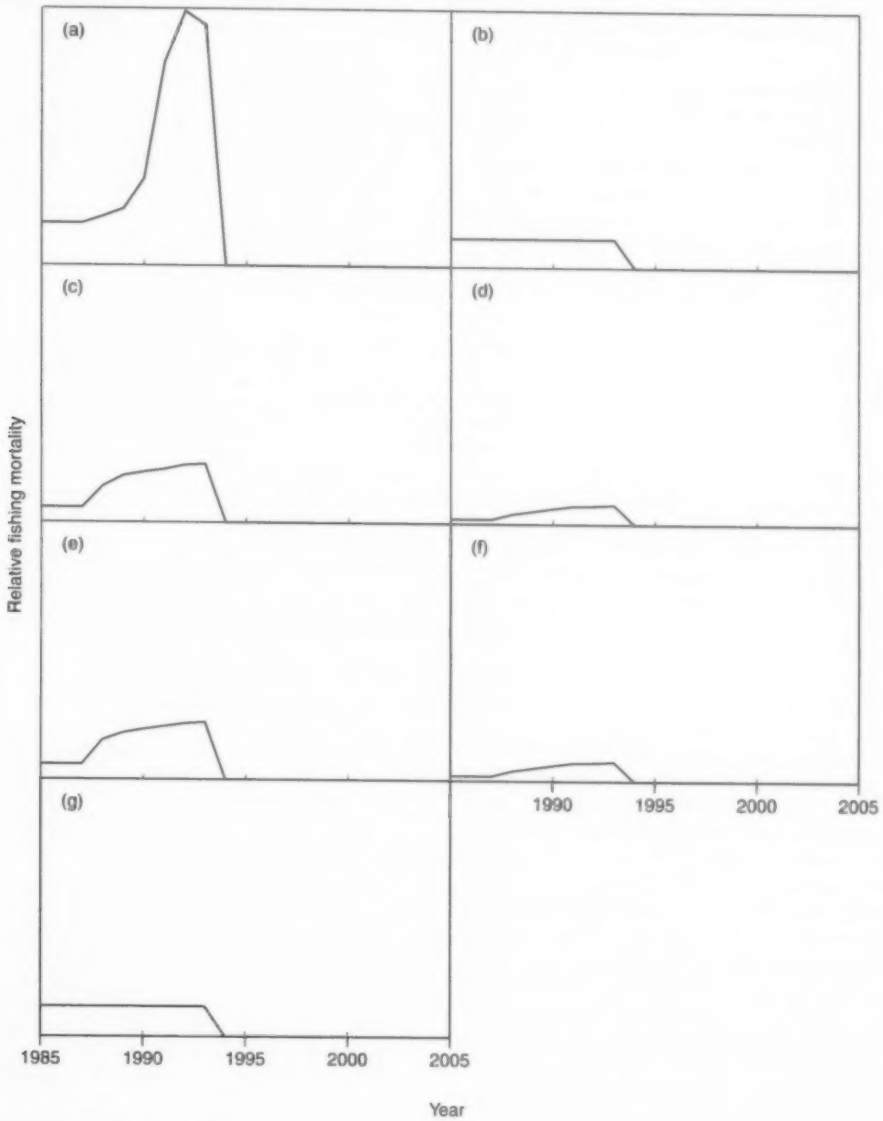
Fig. 1. Five fishing mortality scenarios used in the simulations. (*a*) Scenario 1, cod >35 cm; (*b*) scenario 2, cod ≤35 cm; (*c*) scenario 3, American plaice >35 cm; (*d*) scenario 3, American plaice ≤35 cm; (*e*) scenario 4, Greenland halibut >40 cm; (*f*) scenario 4, Greenland halibut ≤40 cm; (*g*) scenario 5, all other groups.



Although these species do not overlap much spatially, they all feed predominately on small invertebrates (Bundy et al. 2000) and have a similar ecological role, transferring energy from benthic invertebrates to fish. Some commercial species such as cod or American plaice are aggregated by stock, and thus, the cod group consists of NAFO Div. 2J3KL cod and NAFO Div. 3NO cod (Table 1). Of the 17 fish groups, cod, Greenland halibut, and American plaice were split into adults and juveniles. The split group parameters are given in Table 2. The input data for each Ecopath group (catch, biomass, production, consumption, and diet) in Bundy et al. (2000) were taken from studies in the model area for the appropriate time period when possible. For some of the less well-studied species such as sand lance or small demersal feeders, this was not possible and some estimates from the literature were used. For harp seals, cod, Greenland halibut, and American plaice, all data, including diets, were available from the study area. For a full description of all data sources, see Bundy et al. (2000).

The five fishing scenarios used in the Ecosim simulations were based on NAFO catch and effort statistics that were extracted from the NAFO Maritimes Marine Fish Division database. Effort statistics indicate that trawling effort for cod, American plaice, and Greenland halibut did not increase after the mid-1980s, prior to the collapse and closure of the cod fishery (1992 in NAFO Div. 2J3K and 1993 in NAFO Div. 3LNO) and the closure of the American plaice fishery in 1994. However, fishing mortality on these species did increase. In the case of the Northern cod, fishing mortality, estimated from a virtual population analysis (VPA), increased from a value of 0.2 in 1986 (ages 3–10) to 1.8 in 1991 (Murphy et al.

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Table 1. Selected Ecopath groups included in the model showing species composition and, for the main commercial species, stock structure.

Ecopath group	Species composition	Stock structure
Cod >35 cm		2J3KL, 3NO
Cod ≤35 cm		2J3KL, 3NO
Greenland halibut >40 cm		2J3KL(M)NO
Greenland halibut ≤40 cm		2J3KL(M)NO
American plaice >35 cm		2J3K, 3LNO
American plaice ≤35 cm		2J3K, 3LNO
Flounders	Yellowtail flounder	3LNO
	Witch flounder	2J3KL, 3NO
	Winter flounder	
Skates	Thorny skate	
	Smooth skate	
	Winter skate	
	Other skates	
Redfish		2+3K, 3LN, 3O
Large demersal feeders	Hakes (not silver)	
	Haddock	3LNO
	Rocklings	
	Grenadiers	
	Wolffish	
	Eelpouts	
	Common lumpfish	
Small demersal feeders	Gunnels	
	Blennies	
	Pouts and wolfeels	
	Sculpins	
	Alligatorfish	
	Lumpfish (not common)	
	Seasnails	
Capelin		2J3KL, 3NO
Sand lance		
Arctic cod		
Large pelagic feeders	Sharks	
	Tuna	
	Pollock	
	Silver hake	
	Salmon	
Piscivorous small pelagic feeders	Mackerel	NW Atlantic
	Piscivorous mesopelagics	
	Short fin squid	
Planktiverous small pelagic feeders	Herring	
	Atlantic saury	
	Planktivorous mesopelagics	
	Planktivorous squid	
Shrimp		
Large crustaceans		

1997). As in Murphy et al. (1997), it is assumed here that this increase in mortality is due to fishing. American plaice relative fishing mortality, as measured by commercial catch divided by survey biomass, doubled from the mid-1980s to the early 1990s (Morgan et al. 1997). The total mortality of Greenland halibut increased fourfold during this same time period, while catch within the model area declined to around 20% of the 1985–1987 catch levels during the early 1990s (Brodie et al. 1999).

The other gears in the fishery have different patterns of effort. Inshore trawling effort, for example, has declined since the early 1980s. Foreign fishing (mostly trawlers) remained high until 1991 and then rapidly decreased for cod in 1993 and for other species in 1994. Effort by the "other" gears, that is, seine and purse-seine gears, gill nets, set lines, long lines, and traps, increased. The year that increases peaked varied with gear, but ranged from 1989 to 1993, and effort by all gears decreased to essentially zero in 1994.

These effort measures are simply hours fished for trawlers and days fished for the other gear types. They do not account for changes in horsepower, tonnage, improved gear technology, or fish searching technology. Nor do they account for changes in

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Table 2. Additional parameters required by Ecosim for the three groups that are split into two size-classes.

	Age at split[a]	Weight at split (g)	von Bertalanffy *K*
Cod	3	413[b]	0.160[c]
American plaice	8	441[c]	0.076[f]
Greenland halibut	6	586[d]	0.100[g]

[a]See Bundy et al. (2000) for details of the age of split groups.
[b]P. Shelton, Northwest Atlantic Fisheries Centre, St. Johns, NF A1C 5X1, Canada, personal communication.
[c]Brodie et al. (1993).
[d]Bowering and Brodie (1988).
[e]May et al. (1965).
[f]Pitt (1975).
[g]Erzini (1991).

catchability due to changes in fish behaviour, such as range contraction and aggregation. In spite of these caveats, it is assumed that fishing effort reflects fishing mortality, and relative changes in fishing effort are used to approximate relative changes in fishing mortality.

Five fishing scenarios were developed from these fishing mortality and effort data. In scenario 1, the fishing mortality of cod >35 cm was increased from the 1985–1987 value to a value of 1.8 in 1992. In 1994, fishing mortality was decreased to zero 2 years after the cod moratorium. In scenario 2, a uniform fishing mortality of 0.2 was imposed on cod ≤35 cm, assuming that the very low estimate of fishing mortality used in the Ecopath model was an underestimate. Fishing mortality was decreased to zero in 1994. In scenario 3, the fishing mortality of both size groups of American plaice was increased gradually to four times the 1985–1987 value by 1992 and then reduced to zero in 1994. In scenario 4, the fishing mortality of Greenland halibut >40 cm was gradually increased to four times the 1985–1987 value by 1992, and the fishing mortality of Greenland halibut ≤40 cm was increased gradually to two times the 1985–1987 value by 1992. Both were then reduced to zero in 1994 (although in reality, there was a much reduced fishery for Greenland halibut during this time plus a fishery outside the model area; see below). In scenario 5, fishing mortality on all groups other than shrimp, large crustaceans, harp seals, and seabirds was reduced to zero in 1994. In earlier scenarios, the fishing mortality of these groups remained constant at the 1985–1987 level.

Each scenario was run for 20 years and under three assumptions concerning energy flow: bottom-up (flow control factor = 0), top-down (flow control factor = 0.9), and intermediate control (flow control factor = 0.5). During the first 3 years of the simulation, there was no change in fishing mortality, and this represents the stable 1985–1987 model (note, however, that in scenarios 2–5, fishing mortality on cod ≤35 cm was introduced in year 1, 1985). From year 4 onwards (nominally 1988), fishing mortality changes were made, as shown in Fig. 1. The last year of the simulation is nominally 2005.

To investigate the hypothesis that predation by harp seals on cod may be preventing or repressing the recovery of cod, selected simulations were repeated using a slightly modified mass balance model as input. Harp seal abundance has increased by an estimated rate of 5% per annum since the mid-1980s (Shelton et al. 1996). This was modelled in Ecopath by introducing a positive biomass accumulation term of 0.007 $t \cdot km^{-2} \cdot year^{-1}$. In the mass balance model, this has the effect of reducing the other mortality of seals, that is, mortality due to starvation and disease, and indicates that more production is going to population growth. In the simulation model with no perturbation, the harp seal biomass steadily increased.

To check the long-term implications of the scenarios, simulations were also made for 100 years. Walters et al. (1997) found that 50% of the 40 systems that they examined self-simplified when they were perturbed, if top-down control was assumed. They suggested that self-simplification may be caused by inaccurate diet data, which leads to inaccurate estimation of interaction parameters. Reduction in the flow control parameter led to persistence of all groups in these cases.

Results

Intermediate control

As each scenario is introduced under intermediate control, the main impact is on those groups that are subjected to the changes in fishing mortality under each scenario. The results for cod, American plaice, and Greenland halibut are shown in Fig. 2. The biomass of other groups also changed during the simulations, and some of those groups whose biomass changed by more than 30% are also shown in Fig. 2. Under intermediate control, scenarios 1 and 2 led to similar results. Scenario 1 led to a decrease in adult cod, followed by a smaller decrease in cod ≤35 cm. The adult cod biomass then rose rapidly to reach and exceed the 1986 value by around 1995. Cod ≤35 cm regained their 1986 level by around the same time. In scenario 2, both cod >35 cm and cod ≤35 cm declined to a greater extent, and recovery for both groups was slower. Cod >35 cm regained their 1986 biomass by around 2000. As the cod decreased, American plaice and Greenland halibut increased, only to decrease again when cod biomass increased. Several other groups including capelin, shrimp, and large crustaceans also increased and then decreased (Fig. 2).

American plaice biomass decreased as a result of increased fishing mortality in scenario 3. However, the decrease was not large, and they rebounded to increased biomass levels by the late 1990s. The magnitude of the juvenile (≤35 cm) American plaice changes was less than for the adults. The biomass changes of other groups are similar to scenario 2, although the magnitude of the changes is greater, and more groups changed by more than 30%, for example, sand lance (Fig. 2).

In scenario 4, Greenland halibut responded to the increased fishing mortality in a pattern similar to that of American plaice in scenario 3. The impact of these changes in Greenland halibut biomass on the other groups is small, with little difference from the results in scenario 3.

Scenario 5, where fishing mortality on all other commercially fished groups (except shrimp and large crustaceans) was reduced to zero in 1993, had no impact on cod, American plaice, or Greenland halibut. Since their fishing mortality was already reduced to zero, this scenario would not directly affect these groups. However, it also means that there were no impacts on the biomass of these groups from ecological interactions with other species and groups in the model, which may have changed as a result of scenario 5. Skates and large pelagic feeders, for example, increased substantially once fishing mortality was decreased to zero (Fig. 2). Large demersal feeders, flounders, and redfish also increased, while planktivorous small pelagic feeders and small demersal feeders decreased. The biomass changes in other groups such as shrimp and large crustaceans were similar to that described for scenario 4.

The qualitative biomass response of several groups was similar in all scenarios. Generally speaking, the degree of

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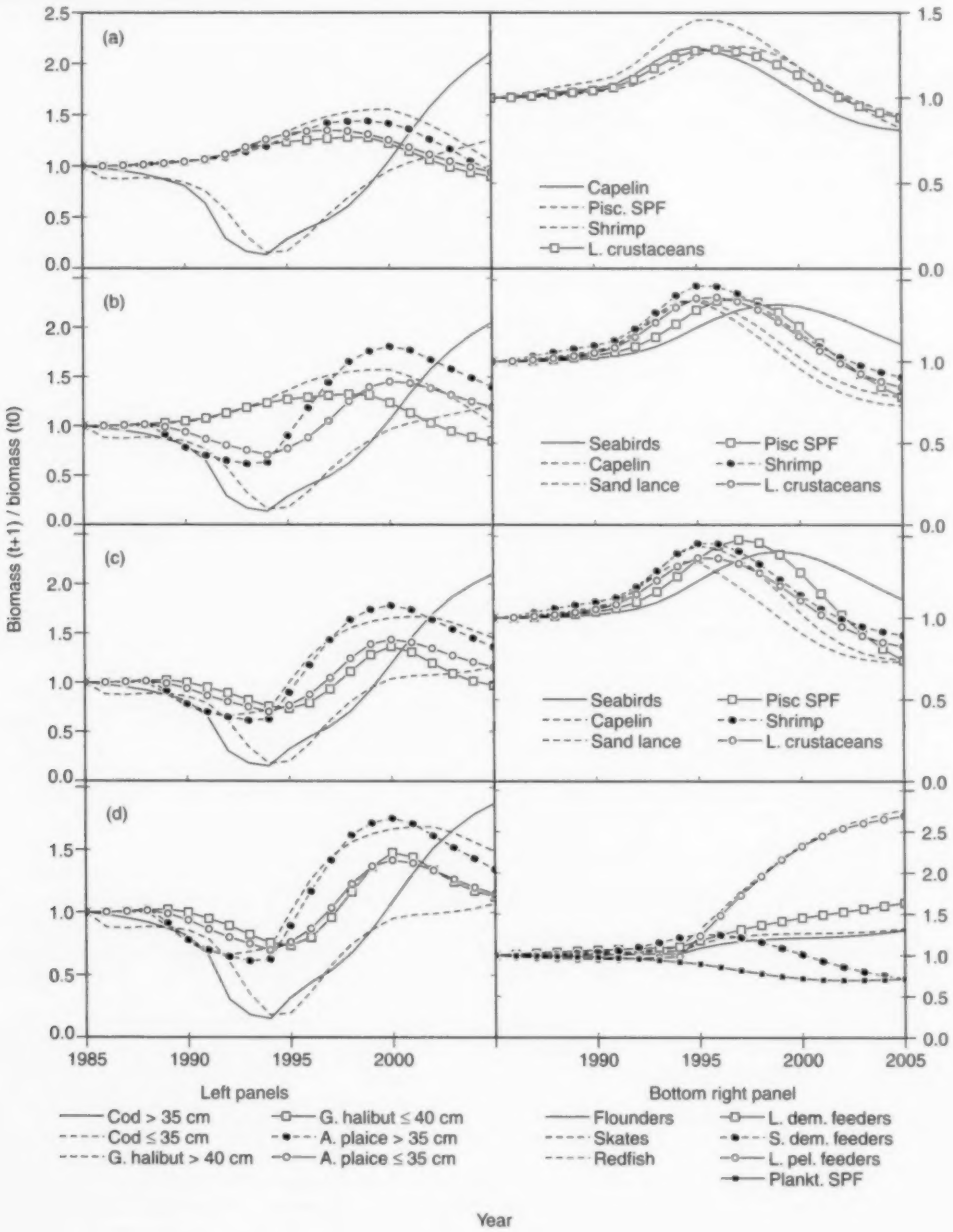
Fig. 2. Comparison of simulation results for scenarios 2–5 using the intermediate control assumption. (*a*) Scenario 2; (*b*) scenario 3; (*c*) scenario 4; (*d*) scenario 5. Results are shown for selected groups whose biomass changed by ±30%. Biomass is expressed as the end biomass/original biomass.



the response increased from scenario 1 to scenario 5. While the cod biomass decreased, the biomass of capelin, sand lance, piscivorous small pelagic feeders, small demersal feeders, shrimp, and large crustaceans increased. They then decreased. For other groups, their qualitative response was similar for all scenarios, with the exception of scenario 5, where significant effects were seen for skates and the large pelagic feeders, for example. The biomass of the marine mammals increased very slightly over the course of all of the simulations. Seabirds increased to around 1996 and then decreased.

Cod, American plaice, and Greenland halibut were affected most by scenarios 1–4 where their fishing mortality was directly changed. The main differences between the scenarios for species other than cod, American plaice, and Greenland halibut were between scenarios 1–4 and scenario

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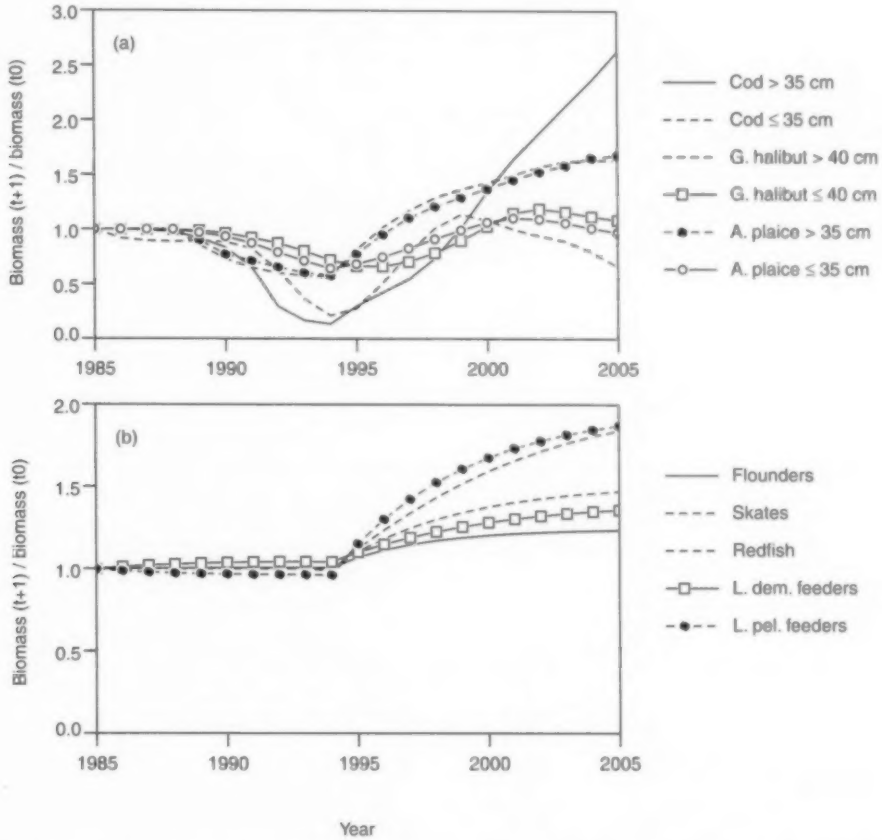
Fig. 3. Results for scenario 5 under the bottom-up control assumption for selected groups whose biomass changed by ±30% plus the flounders. (*a*) Cod >35 cm, cod ≤35 cm, Greenland halibut >40 cm, Greenland halibut ≤40 cm, American plaice >35 cm, and American plaice ≤35 cm; (*b*) flounders, skates, redfish, large demersal feeders, and large pelagic feeders. Biomass is expressed as the end biomass/original biomass.



5. This was also the case for the top-down and bottom-up assumptions (not shown). Since scenario 4 includes increased fishing mortality on cod, American plaice, and Greenland halibut and scenario 5 also includes the fishing mortality changes in other groups, these two scenarios are discussed in the rest of the results.

Bottom-up control versus top-down control

With the bottom-up assumption, there was a relatively flat response to all scenarios by all groups, except those for which fishing mortality was directly changed. The results for scenario 5 are shown in Fig. 3. In all scenarios, the results for cod, American plaice, and Greenland halibut were similar to the intermediate control results shown in Fig. 2, particularly in their decline in response to increased fishing mortality. The recovery rate of cod was a little more rapid, however. In addition, the biomass of cod >35 cm increased to a higher level than under intermediate control, while the biomass of cod ≤35 cm peaked, but decreased again to less than the original biomass by the end of the simulation. American plaice and Greenland halibut recovered at a slower rate than under intermediate control and attained a lower biomass by the end of the simulation.

Scenario 5 had a greater impact on the system than the other scenarios. In addition to the changes described for cod, American plaice, and Greenland halibut, the biomass of skates, redfish, large demersal feeders, and large pelagic feeders increased by more than 30%. Flounders also increased. These are direct responses to changes in fishing mortality. There is minimal response by the other groups in the system, including shrimp and large crustaceans, to these changes in biomass. Bottom-up control represents a system with little interaction between groups.

Top-down control resulted in much higher variations. Figure 4 shows the results under scenarios 4 and 5. The direction of response is largely the same as for intermediate control, but there are important differences. The magnitude of the response is much greater, particularly for cod. The recovery of cod was slower than under intermediate or bottom-up control, and in scenario 5, neither cod >35 cm nor cod ≤35 cm had regained their 1985 biomass by the end of the simulation. On the other hand, there was a greater increase in American plaice biomass during the second part of the simulation than there was in the intermediate and bottom-up simulations: adult American plaice increased to around two times their 1985 biomass by the end of the simulation. The

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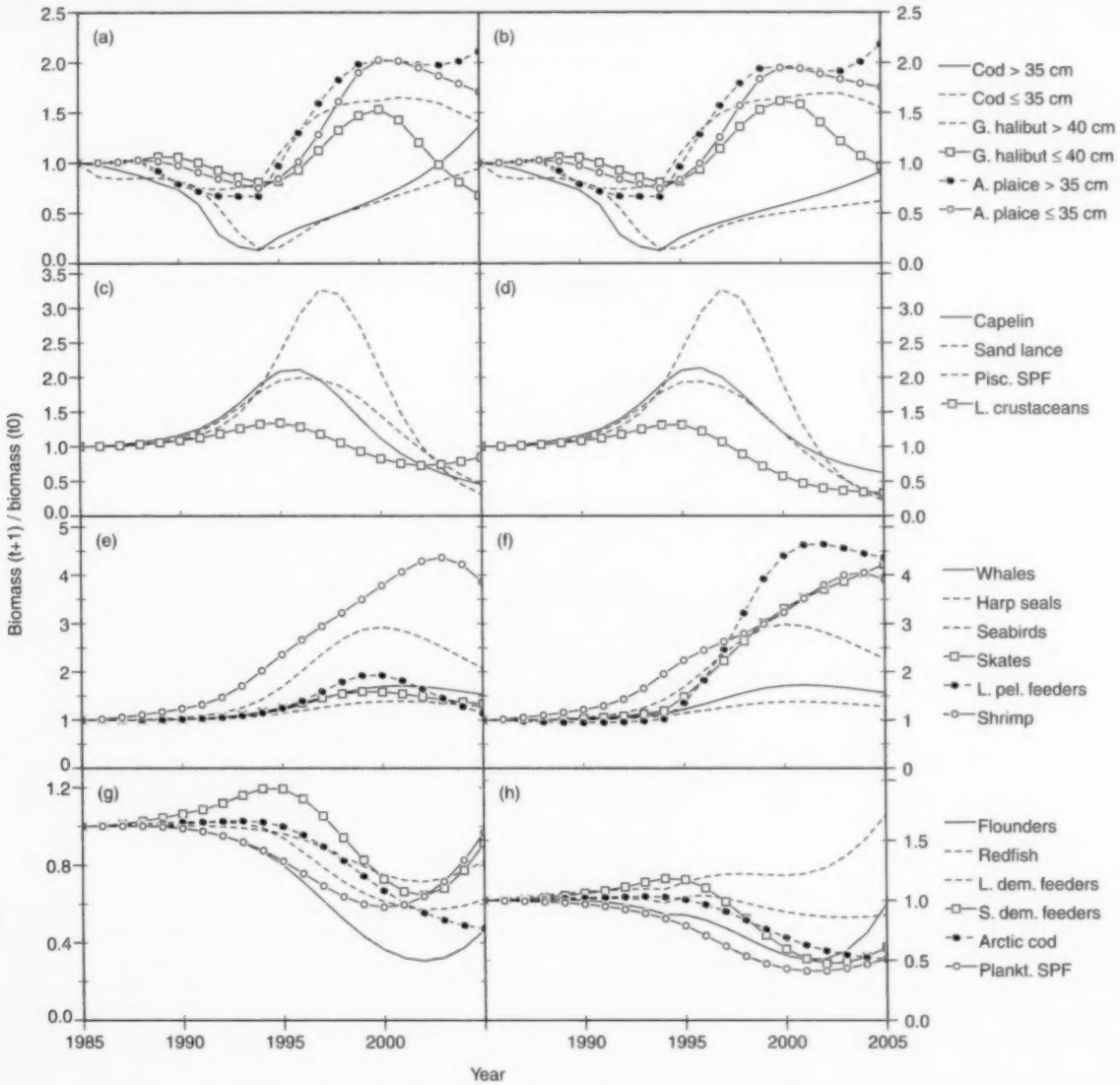
Fig. 4. Comparison of simulation results for (*a*, *c*, *e*, and *g*) scenario 4 and (*b*, *d*, *f*, and *h*) scenario 5 under top-down control assumptions for all groups (excluding the noncommercial invertebrates). Biomass is expressed as the end biomass/original biomass.



response of Greenland halibut to top-down control was similar to their response to intermediate and bottom-up control.

Top-down control increases, and can change, the effect of the perturbations seen with intermediate control. Some groups, such as whales, harp seals, seabirds, shrimp, and skates, undergo a greater increase in biomass. Others undergo a greater decrease, such as the planktivorous small pelagic feeders. Several, for example, capelin, sand lance, piscivorous small pelagic feeders, and large crustaceans, increased and then decreased. However, for other groups, there was also a change in the direction of the biomass trends observed. Flounders and redfish, for example, decreased in scenario 5 under top-down control (Fig. 4), where they had increased slightly under intermediate control (Fig. 2).

Consequences of a 5% increase in harp seal biomass

A 5% increase in the harp seal population was included in the simulations to simulate their rate of increase. The effects of this increase on the initial conditions are shown in Figs. 5*a* and 5*b*. As anticipated, the impact of the harp seals varies with the control assumption. Under the bottom-up assumption, the steady increase in harp seal biomass over the course of the simulation does not affect the results already noted above (not shown). Since under this assumption, each group is modelled as food limited and not predator controlled, this result is expected.

A 5% increase in harp seal biomass does affect the simulation when intermediate and top-down control assumptions are used (Fig. 5). For top-down control, and scenario 5, once

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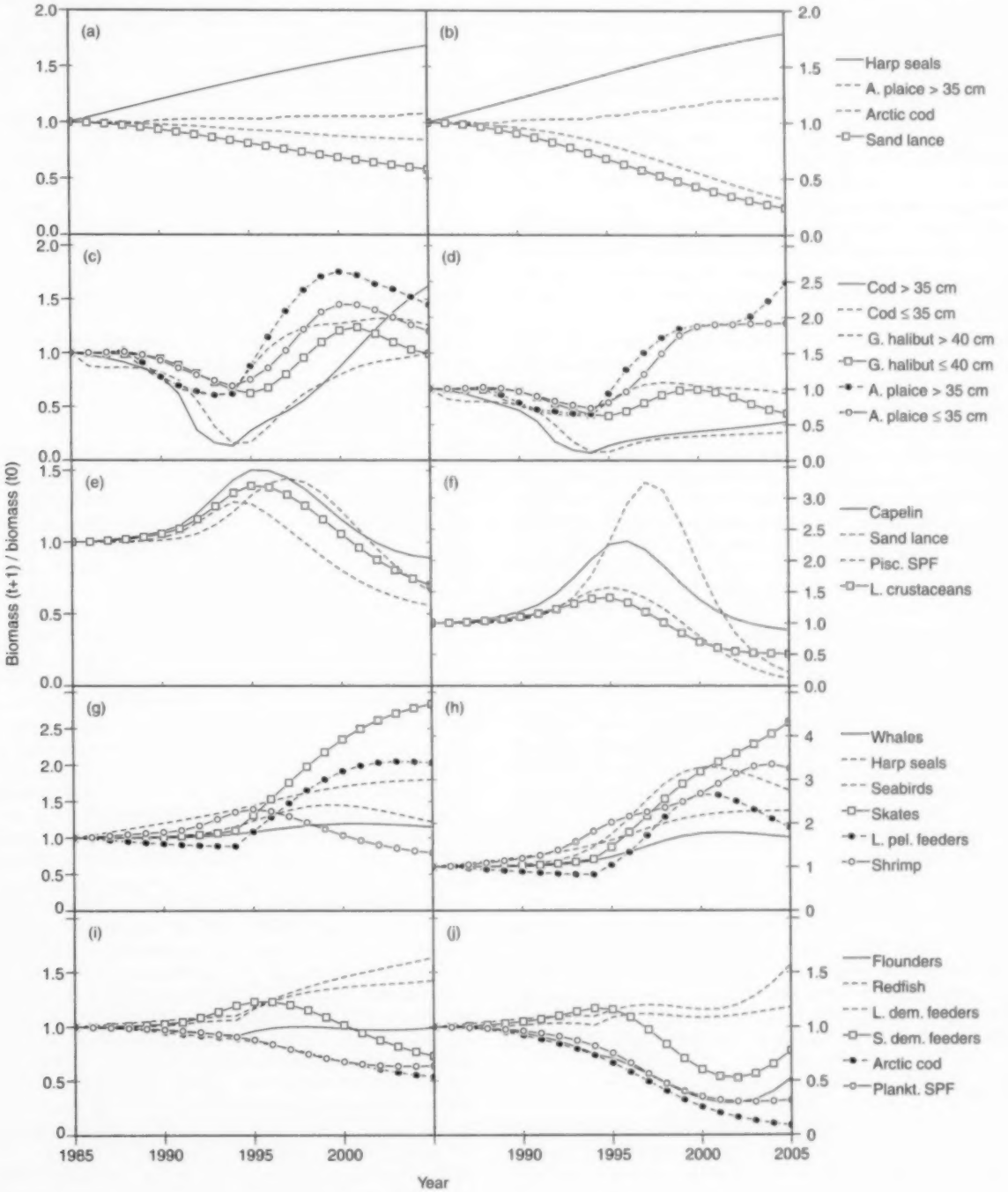
Fig. 5. Effect of a 5% increase in harp seal biomass on the simulations with no change in fishing mortality under (*a*) intermediate control and (*b*) top-down control. Only the groups with the greatest increase and decrease are shown; all other groups fall within these bounds. Also compared are the results of the 5% increase in the harp seal biomass for scenario 5 under (*c*, *e*, *g*, and *i*) intermediate control and (*d*, *f*, *h*, and *j*) top-down control. All groups are shown (excluding the noncommercial invertebrates). Biomass is expressed as the end biomass/original biomass.



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the biomass of cod >35 cm decreased, it only recovered to 50% of the 1985 biomass by the end of the simulation (Fig. 5*d*). The recovery of both size-classes of Greenland halibut was also negatively affected, while American plaice was unaffected. The flounders, small demersal feeders, and Arctic cod showed a very large decrease in biomass, skates increased less, while redfish increased and the large pelagic feeders increased more. Most other groups were largely unaffected by the increase in harp seal biomass, including shrimp and large crustaceans. Similar results were seen for scenario 4. The seals have a greater impact under the top-down control assumption than under the intermediate assumption.

100-year simulations

Scenario 5 simulations were run for an extended period of 100 years, with and without the seal increase. Scenario 5 was chosen, since it produced the greatest perturbation of the system. Consumption to biomass ratios were also checked for consistency.

For intermediate and bottom-up control, the system stabilised by the end of the simulation. For top-down control, there were large fluctuations in biomass throughout the simulation, and some groups were lost. When the seal biomass increase was included in the simulation under top-down control, there was a greater loss of groups. However, all groups still fluctuated at the end of the simulation, and none of these values denote the end point for these groups. If the flow control parameter was reduced, thus reducing the effects of top-down control, no groups were lost.

In the long term, for top-down control, both with and without the harp seal increase and in the absence of fishing, cod biomass increased to 2–2.5 times the 1985 biomass. However, the biomass of American plaice and Greenland halibut was severely reduced. Shrimp biomass was reduced to zero, while the biomass of large crustaceans decreased to about 50% of the 1985 biomass after 70 years and then increased to about 120% of the 1985 biomass.

Estimated consumption to biomass ratios remained within a factor of 2 of the Ecopath input baseline estimates for both the intermediate and top-down assumptions, as recommended by Walters et al. (1997), with the exception of large pelagic feeders and skates for the top-down simulation without the seal population increase. The consumption to biomass ratio of these two groups was reduced to almost one third of the original value. This is related to the large increase in their biomass. The low consumption to biomass ratios hint that the estimated food intake of these groups is unnaturally low. This suggests that for long-term simulations, the assumptions of the model begin to break down. This is not too surprising. The long-term simulation is a long way from the equilibrium assumption where this modelling exercise began.

Discussion

In the following discussion, the biomass trajectories described in the results above are compared with known changes in biomass for some of the key groups in the system. These comparisons are made on a qualitative basis, in terms of the direction, magnitude, and timing of biomass change.

Can the collapse and lack of recovery of the cod and American plaice be replicated?

In all of the simulations, it was possible to model the cod stock collapse in response to high fishing mortality. In some simulations, it was also possible to simulate the poor recovery of cod. The model did not capture the dynamics of American plaice. American plaice biomass did decline in response to increased fishing mortality to around 60% of its 1985–1987 biomass but quickly recovered their 1985 biomass in all simulations.

The cod collapse was well simulated in all scenarios and energy flow control options. The degree of cod recovery varied with the degree of energy flow control. Recovery was slowest under top-down control. The most realistic scenario for cod was scenario 5, with top-down control. Here, cod >35 cm did not regain their biomass by the end of the simulation. Cod ≤35 cm were also considerably reduced and did not recover their biomass by the end of the simulation.

These results were enhanced with the simulation of a 5% increase in the harp seal biomass. In this case, by the end of the simulation, the biomasses of both cod groups were at, or below, 50% of their original value. For the bottom-up and intermediate control assumptions, cod always regained their 1985 biomass by the year 2000, even with the inclusion of seal population growth. In practice, this has not occurred. The simulation most closely matching the current cod population is the top-down control with increasing seal predation.

None of the scenarios or assumptions adequately represented the biomass trends of American plaice. American plaice were subjected to increased fishing mortality between 1985 and 1992 in the simulations, and although their biomass decreased in response to this mortality, the decrease was not substantial. The simulated stock recovered rapidly and regained its 1985 biomass by 1996. In fact, this recovery has not occurred. Bottom-up control simulations, where recovery is more delayed, capture the dynamics of American plaice a little better than intermediate or top-down control.

Raising the fishing mortality of the adult and juvenile groups by 100% could simulate a collapse in American plaice. However, even in this case, the population rebounded very quickly. It is likely that the trophic interactions of American plaice have not been well defined in the model. For example, there are no natural predators of American plaice >35 cm in the model, an unlikely condition. Fishing mortality may also be greater than the value used in the simulations due to discarding and illegal and nonreporting of catch. The Ecopath results indicated a high level of unexplained mortality for American plaice >35 cm, which may be due to nonidentification of predators in the model or an underestimate of fishing mortality. It may also be caused by adverse environmental factors, which are not modelled here. Bowering et al. (1997) suggested that the collapse of the Labrador and northeastern Newfoundland (NAFO Div. 2J3K) American plaice was unlikely to be due to commercial exploitation. Although they did not define a direct cause for the collapse, they indicated that unusual environmental conditions may be involved.

There was some success in the Greenland halibut simulations. The biomass decreased in response to increased fishing mortality and then rebounded to high levels by the mid-1990s in all scenarios except scenario 5 with top-down

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control and a 5% increase in seal biomass. Here, the 1985 Greenland halibut biomass was regained by 1996–1997 and remained at that level for the rest of the simulation. In fact, in NAFO Div. 2J3K, Greenland halibut did regain their 1985 biomass by 1997 and it remains at that level (Brodie et al. 1999). It should be noted that during the 1990s, a substantial fishery developed for Greenland halibut, mainly outside the model area, in the NAFO regulated area of 3LM (outside the 200-mi zone). Although Greenland halibut are assessed as one stock, the NAFO 3LM Greenland halibut was not included in the mass balance model by Bundy et al. (2000), and this catch was not included in the above simulations. If the fishing mortality on large and small Greenland halibut is further increased during 1990–1994 to simulate these large catches, it creates a greater and more realistic initial decrease in biomass and a slower recovery for all control scenarios. This result is consistent with the current knowledge of the Greenland halibut stock: Patterson and de Cárdenas (2000) predicted a slow increase in Greenland halibut biomass after 1994, while Bowering et al. (2000) predicted a faster increase. Both modelling approaches are subject to many uncertainties, as noted by the authors.

There is a negative relationship between cod biomass and American plaice and Greenland halibut biomass in the simulations. The biomass of cod is higher when the biomass of the other two groups is lower, and vice versa. This can been seen by comparing scenario 5 across the bottom-up, intermediate, and top-down control assumptions. All three groups feed on each other's young, as well as their own, so this is an effect of predatory interactions.

Can other ecosystem changes be simulated?

A variable picture emerged for the other species and groups represented in the model. In some cases, the model predicted the general direction of biomass trends since the mid-1980s to 1996, and in others, it failed (see Table 3). Where the model succeeded in qualitatively predicting biomass trends, the best predictions generally occurred using the top-down assumption and scenario 5.

In scenarios 4 and 5 under top-down control, the trophodynamic model predicts that shrimp biomass increases to around three times the 1985 biomass in 1995 and to over four times by around 2002. A more conservative increase is predicted for intermediate control. Here, biomass increases to around 1.5 times the 1985 biomass by 1995 and then decreases. Since the groundfish moratorium, shrimp biomass has increased (Parsons and Veitch 1998).

In all scenarios, under intermediate and top-down control, the large crustaceans are predicted to increase to the late 1990s and then decrease. After the moratorium on cod and American plaice fisheries, greater effort was directed at large crustacean (crab) fisheries in response to a perceived increase in biomass (Dawe et al. 1999; Taylor and O'Keefe 1999). In 2000, quotas were cut by 30% in NAFO Div. 2J3K and 20% in NAFO Div. 3LNO due to a decrease in crab biomass, as predicted by the model.

Interpretation of the results for the flounder group is confounded by the fact that yellowtail and witch flounder have different biomass trajectories after the mid-1990s, while there are no biomass data for winter flounder. The biomass of yellowtail and witch flounder decreased by around 50% of the 1985–1987 biomass by the early 1990s (Bowering and Orr 1997; Walsh et al. 1997). However, the yellowtail flounder stock has increased since 1995 and surpassed the 1985–1987 biomass (Walsh et al. 2000), while the witch flounder stocks have continued to decrease (Bowering 2000*a*, 2000*b*). From 1995 to 1999, yellowtail flounder accounted for over 90% of the biomass of the flounder group. Since both species declined until the mid-1990s, and since after that time, yellowtail flounder has dominated the flounder group biomass, it is assumed that the dynamics of the flounder group represent the dynamics of the yellowtail flounder.

Yellowtail flounder biomass trends were best recreated with scenario 5, under bottom-up and intermediate control, with and without the seal increase. Although the initial decrease in biomass is not captured well, the yellowtail flounder biomass does increase once fishing has ceased. Under top-down control, the flounder biomass showed an initial decline and continued to decline until around 2002.

Under certain conditions, the model can predict the direction and to some extent the timing and magnitude of the biomass dynamics of cod, shrimp, large crustaceans, Greenland halibut, and flounders. The dynamics of other groups are also reasonably captured, under certain conditions. The decrease in biomass of redfish (Power 1995, 1997) and large demersal feeders (Bundy et al. 2000), for example, was predicted. The increase in capelin (Nakashima and Winters 1997) was predicted, and the model also predicted slight increases in the biomass of the harp seals, which have been observed. For some groups, such as hooded seals, sand lance, piscivorous small pelagic feeders, planktivorous small pelagic feeders, large pelagic feeders, whales, and seabirds, we do not know the direction of their biomass trends. These groups are poorly known and are either not well sampled by the bottom trawl surveys or not sampled at all.

For some groups, the predictions were totally wrong. The biomass of Arctic cod increased by about 50% between 1985–1987 and 1992–1994 (Bundy et al. 2000). The intermediate and bottom-up control assumptions did predict a very slight increase or no change in Arctic cod biomass from 1986 to 1995. However, in most scenarios, the top-down assumption led to a decrease in Arctic cod beginning around 1990 and a very large decrease when seal population growth was included in the model. The biomass of skates decreased by around one third between 1985–1987 and 1992–1994 (Bundy et al. 2000),while the predictions here indicated no change.

Do seals have a negative impact on the recovery of cod?

The results shown herein demonstrate that a 5% increase in the harp seal population has a negative impact on the recovery rate of cod under conditions of intermediate and top-down control. There is no effect under bottom-up control. Harp seals have a greater negative impact on juvenile cod than on adult cod, although the recovery of both is almost equally suppressed under top-down control. In the long term, however, the 100-year simulations predicted that the cod biomass would eventually be reestablished whether harp seals increased or not.

Where the model fails

It was not expected that the model would accurately predict the quantitative changes that have occurred in the New-

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Table 3. Conditions for success and failure of the simulations to predict biomass trends in the Newfoundland–Labrador fishery, 1986–1996, under three control scenarios: bottom-up, mixed, and top-down control (✓, success; ×, failure; ?, unknown).

Group	Bottom-up	Mixed	Top-down	Conditions
Whales	?	?	?	Biomass increased in all simulations
Harp seals	×	✓	✓	
Hooded seals	?	?	?	Small biomass decrease in most simulations
Seabirds	?	?	?	Biomass increased in intermediate and top-down simulations
Cod >35 cm	×	×	✓	Top-down, scenario 5, 5% seal population growth
Cod ≤35 cm	×	×	✓	Top-down, scenario 5, 5% seal population growth
American plaice >35 cm	×	×	×	
American plaice ≤35 cm	×	×	×	
Greenland halibut >40 cm	×	×	✓	Top-down, scenario 5, 5% seal population growth
Greenland halibut ≤40 cm	×	×	✓	Top-down, scenario 5, 5% seal population growth
Flounders	✓	×	×	
Skates	×	×	×	
Redfish	×	×	✓	
Large demersal feeders	×	×	✓	Top-down, scenario 4
Small demersal feeders	×	×	×	
Capelin	×	✓	✓	
Sand lance	?	?	?	Biomass increased in intermediate and top-down simulations
Arctic cod	×	✓	×	Intermediate control indicated a slight increase
Large pelagic feeders	?	?	?	Biomass increased in scenarios 4 and 5
Piscivorous small pelagic feeders	?	?	?	Biomass increased in intermediate and top-down simulations
Planktivorous small pelagic feeders	?	?	?	Biomass decreased in intermediate and top-down simulations
Shrimp	×	✓	✓	
Large crustaceans	×	✓	✓	
Number of successes	1	5	10	
Number of failures	15	11	6	
Number of unknowns	7	7	7	

foundland Labrador fishery, but rather that it would indicate the direction, timing, and magnitude of changes in biomass. To this extent, the model has been reasonably successful for several groups, such as cod, shrimp, and large crustaceans. The successes and failures of the model are listed in Table 3. The top-down control assumption produced the most successful results, followed by mixed control. The lack of success under the bottom-up control assumption suggests that bottom-up control is not the appropriate form of energy control for most of the groups in this system. As noted above, there is ongoing debate concerning the processes that govern flow control in the oceans. In reality, different groups in the ecosystem are governed by different forms of energy control, and it is likely that a melange of flow control patterns exist. Greater success across more groups may be obtained by varying assumptions about energy control for different components of the ecosystem.

Under top-down control, the model correctly predicted biomass changes for 10 groups and was wrong for six groups. For the other seven groups, the actual pattern of biomass change, if any, is unknown. Christensen (1998) predicted the biomass changes observed in the Gulf of Thailand using this approach and concluded that the effects of fishing were paramount in this ecosystem. Bundy (1997) was able to replicate some of the biomass changes that had occurred in San Miguel Bay, the Philippines, by simulating changes in fishing effort by the different sectors in the fishery. In both cases, fishing was the prime determinant of change, not environment. It can also be concluded from the results presented here, with some provisos, that fishing was the main cause of change for several species such as cod and possibly Greenland halibut. Other species such as large crustaceans and shrimp have increased as a result of the decrease in cod biomass and trophic interactions

This conclusion assumes that the fishing mortalities used in the simulations are representative of actual fishing mortalities. This may not be the case. The standard practice of partitioning total mortality into an assumed natural mortality of 0.2, with the rest going to fishing mortality, is being revised. It has recently been shown that there is substantial annual variation in the natural mortality of eastern Scotian Shelf cod (Caihong Fu, Department of Fisheries and Oceans, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada, personal communication). In a recent VPA for American plaice, Morgan et al. (1999) determined that assuming a natural mortality of 0.6 on all age groups from 1989 to 1996 produced the best fit. The severalfold increase in fishing mortality assumed for cod may not therefore be all attributable to fishing mortality: some of the increase may be due to natural mortality caused by other factors, such as environmental changes. However, although attempts to vary natural mortality in order to obtain a good VPA fit have been made for northern cod, an acceptable VPA has not been produced since the 1997 assessment. Thus, the fishing mortality estimates given in the 1997 assessment were taken at face value and used here, providing the conclusion above.

For some species such as cod or skates, there is also likely to be more fishing mortality than the model allowed for due

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to misreporting of catch and by-catch, discarding, and illegal fishing. For example, the decline of skates is better simulated when their fishing mortality is increased. Noncommercial species such as the small demersals may be caught as by-catch in commercial fisheries and thus incur fishing mortality, which is not recorded. Thus, a more realistic representation of fishing mortality for the minor commercial and noncommercial species may produce better biomass simulations for these groups.

The aim of this analysis was to determine if fishing and trophic interactions, using our current knowledge of fishing mortality, could account for the observed ecosystem changes. There was thus no attempt made to model changes in natural mortality due to environmental effects or to model other forms of mortality, for example, greater fishing mortality. While the model was successful for some groups, the failure of Ecosim to simulate the biomass trends of all groups indicates that other factors such as unaccounted natural mortality are likely to be important.

Ecosim is a trophodynamic model, and it is not possible to directly explore other causes of mortality and their effects within its framework. However, there have recently been some modifications made to Ecosim in order to indirectly explore environmental effects. For example, the productivity of a group can be increased or decreased using forcing functions, and the recruitment function can be modified to simulate poor recruitment. Environmental effects may explain why the model failed to simulate the prolonged lack of recovery of American plaice or the increase in Arctic cod.

One mechanism for low productivity or low recruitment success may be reduced age structure of exploited fish populations. Longhurst (1998) suggested that the long-lived age structure and delayed age of maturity of northern species such as cod and American plaice is an adaptation to recruitment variability that is best fitted to their long-term survival. Removal of that age structure endangers their ability to survive poor recruitment. Cod, American plaice, and Greenland halibut have all lost the older age groups and thus a large part of their spawning potential. Not only would this cause collapse, it would also seriously retard, if not prevent, recovery (Longhurst 1998). This reduction in spawning potential was not modelled in Ecosim but could be modelled indirectly using the recruitment function. Another cause of poor recruitment may be poor fish condition and the suppression or reabsorption of eggs before spawning.

In addition to environmental effects, the failure of Ecosim to model all groups successfully may be due to model structure. Walters et al. (1997) identified the equilibrium assumption of Ecopath as "the most worrisome" weakness of Ecosim. As the model is moved from the mass balance, such as by reducing fishing mortality to zero, it is moved away from the mass balance situation for which the Ecopath parameters were appropriate. Thus, the extrapolation becomes more tenuous. It would be appropriate to assemble a second Ecopath model for the postcollapse period using direct biological parameter estimates to determine the extent of change in the assumptions of the first model.

While there is scope to further develop and improve the Ecosim model of the Newfoundland–Labrador Shelf, it is concluded that the trophodynamic simulation model presented here is consistent with the theory that the Atlantic cod fisheries of Newfoundland and Labrador collapsed due to excess fishing mortality. In addition, it correctly predicted the increased biomass of shrimp and large crustaceans. This result is consistent with the hypothesis that these species increased in abundance as a result of the collapsed biomass of cod and reduction in predation. The results also support the hypothesis that the recovery of cod is currently being retarded by the increased biomass of harp seals due to predation by harp seals on cod.

While limitations of the model in some areas are acknowledged, this type of ecological approach is necessary if we are to achieve a greater understanding of the ecosystems upon which our fisheries are based. A well-founded ecological understanding of marine systems is essential if we are to improve fisheries management and to achieve sustainable resource use.

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Appendix

Table A1. Input parameters and estimates from the Ecopath model of Newfoundland–Labrador, 1985–1987, presented in Bundy et al. (2000).

Group	Biomass ($t \cdot km^{-2}$)	*P/B* ($year^{-1}$)	*Q/B* ($year^{-1}$)	EE	Production/ consumption	Harvest ($t \cdot km^{-2}$)	Trophic level
Whales	0.251	0.100	11.794	0	0.008		4.2
Harp seals	0.184	0.102	17.412	0.053	0.006	0.001	4.4
Hooded seals	0.034	0.109	13.100	0	0.008		4.7
Seabirds	0.012	0.250	54.750	0.333	0.005	0.001	4.2
Cod >35 cm	2.044	0.651	2.200	0.883	0.173	0.603	4.2
Cod ≤35 cm	0.275	1.597	6.090	0.950	0.262		3.9
Greenland halibut >40 cm	0.348	0.296	1.478	0.620	0.200	0.035	4.5
Greenland halibut ≤40 cm	0.471	0.872	4.476	0.950	0.195	0.002	4.3
American plaice >35 cm	0.972	0.472	2.000	0.218	0.236	0.100	3.7
American plaice ≤35 cm	0.784	0.625	3.736	0.456	0.167	0.025	3.7
Flounders	1.092	0.372	3.600	0.950	0.103	0.079	3.1
Skates	0.258	0.286	2.878	0.528	0.099	0.037	4.0
Redfish	1.598	0.330	2.000	0.950	0.165	0.178	3.7
Large demersal feeders	0.845	0.262	1.747	0.676	0.150	0.052	3.4
Small demersal feeders	2.380	0.400	2.000	0.950	0.200		3.1
Capelin	13.289	1.145	4.300	0.849	0.266	0.126	3.3
Sand lance	2.310	1.150	7.667	0.950	0.150		3.2
Arctic cod	2.807	0.402	2.633	0.950	0.153		3.4
Large pelagic feeders	0.029	0.400	3.333	0.950	0.120	0.006	4.2
Piscivorous small pelagic feeders	1.405	0.600	1.767	0.950	0.340	0.015	4.1
Planktivorous small pelagic feeders	3.167	0.500	3.333	0.950	0.150	0.019	3.3
Shrimp	0.816	1.450	9.667	0.950	0.150	0.004	2.5
Large crustaceans	1.732	0.450	4.420	0.950	0.102	0.016	2.9
Echinoderms	112.300	0.600	6.667	0.062	0.090		2.0
Molluscs	42.100	0.570	6.333	0.083	0.090		2.0
Polychaetes	10.500	2.000	22.222	0.280	0.090		2.0
Other benthic invertebrates	7.8	2.500	12.500	0.306	0.200		2.0
Large zooplankton	20.361	3.433	19.500	0.950	0.176		2.6
Small zooplankton	30.464	8.400	20.667	0.950	0.406		2.0
Phytoplankton	26.860	93.100		0.311			1.0
Detritus	389			0.589			1.0

Note: Shown are *P/B* (production to biomass ratio), *Q/B* (consumption to biomass ratio), EE (ecotrophic efficiency), the proportion of production that is used within the system, harvest, and fractional trophic levels (Christensen and Pauly 1992).

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Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid

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Abstract: We evaluated the depletion and recovery rates of sperm number and vas deferens weight following mating for male *Callinectes sapidus* both below (<127 mm carapace width) and well above (>140 mm) the fishery size limit for hard crabs in Chesapeake Bay (127 mm). Large males had low sperm count and vas deferens weight immediately after mating and required approximately 9–20 days to fully recover. After mating, small males had significant reduction in sperm number despite no significant change in vas deferens weight. Furthermore, small males with completely recovered seminal stores had significantly lower vas deferens weight than fully recovered large males but did not differ significantly from large males in number of sperm. The changes in vas deferens weight and sperm count following experimental mating suggest that large males delivered 21 times as much seminal fluid and 2.25 times as much sperm as small males. Field collections in a subestuary of Chesapeake Bay revealed that the majority (50–90%) of males had extremely low vas deferens weight relative to males with fully recovered sperm volume. Since the fishery targets males primarily, reducing both the number and average size of males in the population, many females may be mated with small or recently mated males that transfer less seminal material.

Résumé : Nous avons évalué les taux d'épuisement et de récupération du nombre de spermatozoïdes ainsi que la masse du vas deferens après l'accouplement chez des Crabes bleus, *Callinectes sapidus*, mâles de tailles inférieure (<127 mm de largeur de carapace) et bien supérieure (>140 mm) à la taille minimale de capture dans la baie de Chesapeake (127 mm). Chez les mâles de grande taille, le nombre de spermatozoïdes et la masse du vas deferens sont faibles immédiatement après l'accouplement; ils mettent environ 9–20 jours à revenir complètement à la normale. Après l'accouplement, les mâles de petite taille ont une réduction significative du nombre de leurs spermatozoïdes sans avoir de changement significatif dans la masse de leur vas deferens. De plus, après la récupération complète des réserves de spermatozoïdes, les petits mâles ont le vas deferens moins lourd que les grands mâles, sans qu'il y ait de différence significative entre les deux groupes dans le nombre des spermatozoïdes. Une étude du changement de masse du vas deferens et du nombre des spermatozoïdes après des accouplements expérimentaux laisse croire que les grands mâles libèrent 21 fois plus de liquide séminal et 2.25 fois plus de spermatozoïdes que les petits mâles. Des échantillons recueillis dans un sous-estuaire de la baie de Chesapeake indiquent que chez la majorité (50–90%) des mâles, la masse du vas deferens extrêmement faible par comparaison à des mâles ayant pleinement récupéré leurs réserves. Puisque la pêche cible surtout les mâles, ce qui réduit tant le nombre que la taille moyenne de ceux-ci dans la population, plusieurs femelles doivent s'accoupler avec de petits mâles ou avec des mâles qui viennent de se reproduire et qui libèrent moins de semence.

[Traduit par la Rédaction]

Introduction

The reproductive contribution made by males is rarely examined when determining stock–recruit relationships. Sperm and seminal fluid are usually considered to be both energetically inexpensive for males to produce and easy to distribute to many females. The reproductive potential of a population is often estimated by counting mature females and, in managed species, spawning stocks are frequently maintained by allowing females to reach maturity prior to harvest, ignoring paternal influences altogether. Recent research suggests that this approach of assuming ample sperm and (or) seminal

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fluid resources may be an oversimplification of the reproductive system in many decapod crustaceans (Ennis 1980; Smith and Jamieson 1991; Paul and Paul 1992). In many managed species in which only female spawning stock is protected, the consequence of overharvesting males can be a reduction in reproductive potential via sperm limitation or insufficient seminal fluid.

The male contribution to reproduction includes both sperm and seminal fluid. If sufficient numbers of spermatocytes are not available to fertilize a female's egg production, sperm limitation will result. Furthermore, insufficient availability of seminal fluid may also limit reproductive potential. Seminal fluid is not only the medium in which sperm is transferred but may also be necessary for successful sperm storage. For example, the seminal plasma of some brachyuran crabs contains a large amount of organic substances used for sperm metabolism (Subramoniam 1991). In addition, it has been shown that proteins found in seminal fluid play a critical role in effective sperm storage in *Drosophila melanogaster* (Tram and Wolfner 1999) and even act as antibacterial agents that may protect the reproductive tract of the mud crab, *Scylla serrata* (Jayasankar and Subramoniam 1999). Seminal fluid also plays a role in forming a "sperm plug" that may function to prevent sperm from leaking out of a female once deposited and (or) to block competing males from depositing additional sperm (Ryan 1964; Hartnoll 1969; Sainte-Marie and Sainte-Marie 1999).

Certain characteristics of a population's reproductive strategy suggest an inherent possibility of sperm and (or) seminal fluid limitation. For example, sperm limitation may occur in systems in which females fertilize multiple broods or large numbers of eggs using sperm stored from one mating event. Females may simply run out of sperm, as is the case for large American lobsters (*Homarus americanus*) that spawn twice in the same instar (Waddy and Aiken 1986), or female tanner crabs (*Chionocetes bairdi*) that do not receive sufficient sperm to fertilize multiple broods when inseminated only during their molt to maturity (Paul and Paul 1992).

Mating systems in which females must store sperm for a long time between copulation and fertilization may also be sperm and (or) seminal fluid limited. Sperm viability may decline over time, especially if energy sources for metabolic requirements of sperm are insufficient. Seminal plasma has been shown to serve the nutritional requirement for metabolism of stored sperm in such species as the crab *S. serrata* (Subramoniam 1993). The transmission of adequate seminal fluid may be critical for maintaining sperm viability.

Populations that have a low density of males with high reproductive fitness may also be susceptible to sperm limitation. Reduced reproductive fitness in males may result when poor health or some other physiological constraint, such as molting or immaturity, results in decreased sperm and (or) seminal fluid production or transfer capability. High mating frequency of males also may reduce a population's reproductive potential. Females receive less sperm or seminal fluid if males mate more frequently than reproductive resources can be replenished. These males would deliver reduced quantities of sperm and (or) seminal fluid to their second and subsequent mates. For example, large male king crabs (*Paralithodes camtschatica*) show a reduction in fertilization success after nine successive matings (Powell et al. 1973). Fertilization success may also be lower in small mature males that do not produce or transfer as much sperm and (or) seminal fluid as large mature males. In contrast with large male king crabs, small males show a reduction in fertilization success after only seven successive matings (Powell et al. 1973). In the crab *S. serrata*, there is an increase in spermatophore size as well as in the concentration of inorganic ions and organic substances in seminal fluid as males mature (Subramoniam 1993).

Populations with a low density of males (in any reproductive condition) may also be sperm limited. Under this condition, females may have to wait some period of time to find a mate, leaving their reproductive potential unfulfilled for that period. When densities of males are extremely low, females may not be able to find a mate at all. Large female American lobsters and Dungeness crabs (*Cancer magister*) probably experience difficulty finding suitable mates in areas where the fishery intensively exploits males (Ennis 1980; Smith and Jamieson 1991). This situation can be aggravated if the sexual receptivity of females is temporally limited. For example, female king crabs and snow crabs (*Chionocetes opilio*) are sexually receptive for only a few days each year (Sainte-Marie and Lovrich 1994).

Mating systems with one or more of these elements provide effective models with which to investigate sperm limitation. Blue crabs, *Callinectes sapidus*, have many of these attributes: males can mate repeatedly, females mate only once during a 2- to 3-year life span, females are sexually receptive only during their molt to maturity (~1 week during their entire lifespan), reproduction is highly seasonal at the population level (most mating in the Chesapeake Bay occurs during bursts in the spring and summer), and females use stored sperm and seminal fluid from an individual male's ejaculate over a period of several months to fertilize multiple broods of ca. $1–4 \times 10^6$ eggs each (Millikin and Williams 1980; Prager et al. 1990).

The likelihood that limiting conditions will arise in *C. sapidus* is aggravated by the effects of an intense fishery. In Chesapeake Bay (U.S.A.), the fishery has a minimum size limit (127 mm carapace width (CW)) as well as restrictions on the harvest of some ovigerous females and, hence, selectively removes large males from the population. A study during Maryland's summer fishery suggests that fishery pressure may be 3–6 times higher on males than on females (Casey et al. 1990). Remaining small males that escape the fishery are left to mate with the still relatively unfished female population (mature females are also fished from the population, but only after sperm resources are allocated). In addition, a monitoring program in mid Chesapeake Bay indicates that average male size has been declining over the past 30 years as pressure from the fishery has increased, whereas female size has shown no such decline (Abbe and Stagg 1996). Ejaculate size is positively correlated with male size (see Results; Jivoff 1995); therefore, although females in the population are presumably capable of maintaining normal levels of egg production (egg production is correlated with female size; Prager et al. 1990), they may actually be fertilizing fewer eggs with a reduced quantity of sperm and (or) seminal fluid received from fewer and smaller males.

It should be possible to determine if sperm limitation is occurring in this system by measuring ejaculate size

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(amount of sperm and seminal material that females receive), estimating lifetime egg production for females, and determining if egg production exceeds the fertilization potential of sperm resources; however, researchers have had difficulty keeping mature females under suitable brooding conditions for long enough to obtain reliable estimates of lifetime egg production and fertilization rates (Sulkin et al. 1976; Darsono 1992).

Instead, we chose to explore the potential for sperm and (or) seminal fluid limitation in this system by identifying underlying mechanisms that could bring about the limitation. Operational sex ratios reveal whether male abundance is low, thus potentially inducing limiting conditions. Assessing this ratio involves not only counting the overall numbers of males and receptive females, but also identifying variables that affect competition for mates, such as the size, condition, and molt stage of males. It is also possible to determine if sperm limitation is likely to occur by examining the reproductive condition of males in areas where mating occurs. If most males have abundant seminal resources, sperm limitation is unlikely. Conversely, if many males in the population have low sperm and (or) seminal fluid volume, females mated with these males may experience reduced fertilization success. Because of the fishery's influence on the size distribution of males in the population, it is also important to determine the relationship between size and the reproductive potential of males, as well as to estimate the replenishment rates of sperm number and seminal fluid following mating for males of different sizes.

The purpose of this study was to determine the likelihood that sperm limitation occurs in a Chesapeake Bay population of *C. sapidus* by (*i*) determining the influence of male size and mating history on their reproductive potential, (*ii*) determining the temporal limitations for replenishment of sperm number and seminal fluid following mating for males larger and smaller than the fishery size limit, and (*iii*) determining the reproductive condition of males in an area where mating occurs.

Materials and methods

All experiments were conducted at the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland, U.S.A. (38°51′N, 76°32′W) (Fig. 1). SERC is located on the Rhode River, a subestuary draining into northwest Chesapeake Bay. Salinity in the Rhode River ranges from 5 to15 psu (practical salinity units) from spring to fall and the temperature ranges from 0 to 29°C from winter to summer.

Reproductive resources of males: the role of size and mating history

We compared the reproductive condition of small and large male crabs, as well as the recovery rates of sperm stores for crabs above and below the fishery size limit (127 mm CW). Male blue crabs were collected by crab pot in the Rhode River and separated into large (>140 mm CW) and small (115–127 mm CW) size-classes. Size-class limits were selected to ensure that all individuals in the small size-class were mature (males reach maturity at ~110 mm CW; Van Engel 1990) but below the current harvestable size limit for the blue crab fishery in Maryland. The large size-class limit (>140 mm CW) was selected to provide a contrast group well above the legal limit of the fishery. Only intact intermolt (hard-shelled; molt stage C) animals were used in these experiments.

To permit the recovery of sperm resources, males were held in large submerged cages near the SERC dock for 20 days prior to experimentation. Preliminary research indicated that this provided sufficient time for seminal resources that might have been spent prior to collection to be replenished. They were fed a mixed diet of fish and molluscs placed in the enclosures each day. The amount of food offered was based on estimated daily feeding rates for crabs of this size (Nye 1989). At the conclusion of the recovery period, crabs were assessed for molt stage and general condition (e.g., missing limbs, parasites, etc.). We assumed that the enclosures provided adequate habitat for growth and behavior, because mortality was low (<10% during recovery periods) and most crabs were found to be in good condition (active and intact).

Following the initial holding period, we measured the recovery rate of vas deferens weight and sperm number for both size-classes of males following copulation. Animals with fully replenished sperm resources were split into five groups. The first group consisted of control (unmated) males. Individuals in the remaining groups were mated twice in close succession to deplete their sperm levels to nearly zero. Receptive females in the advanced premolt stage (D_3) of the prematurity instar, termed "pubertal," were obtained from commercial shedding operations near the study site. In most cases, males mated twice in 2–4 days if offered a D_3 molt stage pubertal female after their first mate was removed (D_3 females molt or are receptive to mating in 0–2 days). For large males (>140 mm), groups of mated male crabs were then sacrificed 0, 3, 9, and 20 days after their last mating and assessed for reproductive condition by weighing their vasa deferentia and performing sperm counts. Food was supplied ad libitum during recovery periods. Crabs below the legal size limit of the fishery (127 mm) were assessed for reproductive condition prior to mating and at 0 and 3 days after they had mated twice in close succession (but not at 9 and 20 days).

The total number and concentration of sperm were determined for each male. Following determination of wet weight, the entire vasa deferentia and their contents were homogenized in 2 mL of artificial seawater (30 psu) and stained using 7–8 drops of 1% gentian violet. Samples were then passed through a 35-µm filter, to separate large bits of ruptured spermatophores from sperm cells. Both the filter and filtrate were examined to ensure that all spermatophores were ruptured and all sperm were passing through the filter. Filtrate volume was raised to 100 mL/g of vas deferens with 30 psu artificial seawater, to dilute sperm sufficiently to avoid their overlapping each other in the counting chamber. Ten microlitres of the evenly mixed suspension was placed on each of two Petroff-Hausser spermacytometers and allowed to settle for 5 min. Sperm were counted under phase contrast at 400× magnification and low–medium light. If the two cell counts differed by >10%, the original sample was resuspended and the procedure performed again. This approach minimized variability in the sperm count data due to sampling technique. The total number of sperm for each male was then calculated by dividing the number of cells counted by the volume counted and multiplying this quotient by the total dilution volume ((number of cells counted/volume counted) × total dilution volume). Sperm concentration was determined for each individual by dividing the total number of sperm by the weight of the vasa deferentia.

Reproductive condition of male blue crabs in the Rhode River

Crabs were sampled by trawl from a variety of habitats and depths throughout the Rhode River to determine the reproductive condition of the population during the mating season (June–October) in 1996 and 1997. Otter trawls (7 mm cod end) were conducted three times per month in each of four sites (Fig. 1): a shallow (~2 m) mud-bottom site at the head of the subestuary, a sand-bottom site (~2 m) at the mouth of the subestuary, a ~4 m deep mud-bottom site at the mouth

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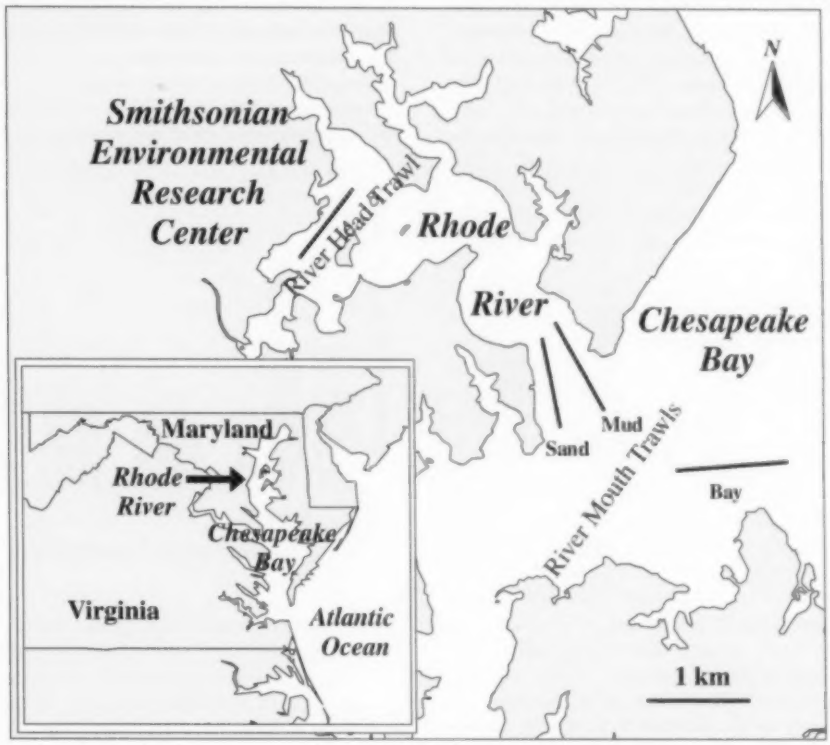
Fig. 1. Trawl locations within the Rhode River subestuary. Inset shows the location of SERC on Chesapeake Bay (38°51′N, 76°32′W).



of the subestuary, and a deep (~4 m) site in Chesapeake Bay off the mouth of the Rhode River. While no sampling strategy is perfectly representative of a population, our sampling design captured a suitable cross section of blue crabs in the Rhode River for our study, since it incorporated multiple habitat types used by crabs and locations from the head of the subestuary to its opening into Chesapeake Bay (Hines et al. 1987). This sampling strategy enabled us to (*i*) estimate the overall reproductive condition of male blue crabs in the Rhode River, (*ii*) determine patterns in reproductive condition based on location in the Rhode River, (*iii*) assess temporal differences in reproductive condition between and within reproductive seasons, and (*iv*) estimate the operational sex ratio in the Rhode River.

Size, sex, molt stage, and autotomy status (intact vs. missing or regenerating limbs) were recorded for each crab. The reproductive condition of male crabs was determined by weighing the vasa deferentia. Scatter plots of vas deferens weight versus CW were generated from the data for each month. Data for all locations were pooled in each month, because there were no location effects (see Results). Sperm counts could not be conducted on field samples, owing to time constraints.

The average weight of vasa deferentia for crabs mated a single time following complete recovery of sperm resources was determined and was used as a reference point with which to compare the reproductive condition of field-collected males. Mean vas deferens weight was obtained by holding a group of 15 males, whose sizes covered a large range (115–160 mm CW), for 20 days (resulting in complete recovery of sperm resources as described above), allowing them to mate a single time, and then weighing the vasa deferentia. The proportions of trawl-caught crabs that lay above and below this value were classified as being either "recovered" or "depleted," respectively. The reproductive condition of a control group of males held under identical conditions but prevented from mating was also determined, to provide a reference level for a "fully recovered" male.

Statistical analyses

Vas deferens weight, sperm-count, and sperm-concentration data from recovery-rate experiments were log-transformed to meet assumptions of normality and homoscedasticity during tests of significance. Vas deferens weight, sperm count, and sperm concentration were compared among groups, using *t* tests for pairwise comparisons (e.g., large unmated vs. small unmated) and the differences of least squares means (LSD) procedure for comparisons involving multiple means (Zar 1996). Field data were analyzed to identify temporal and spatial differences in male reproductive condition, by comparing mean vas deferens weights for each month and location using a mixed-model analysis of covariance ($\alpha = 0.05$) (year was analyzed as a random variable, while location, autotomy status, and molt stage were considered fixed) with a LSD mean comparison procedure using crab size as a covariate (preliminary analysis revealed that crab size had a significant influence on vas deferens weight). Differences in vas deferens weight based on autotomy status (intact vs. missing or regenerating limbs) and molt stage (A, early post molt; B, late post molt; C, intermolt; D, premolt; E, ecdysis) were examined in the same way.

Results

Reproductive resources of males: role of size and mating history

Small unmated males in the experimental group had a significantly lower mean vas deferens weight than large unmated males (1.41 vs. 4.23 g, $t = 3.60$, $p < 0.001$; Fig. 2).

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Fig. 2. Vas deferens weight of small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.

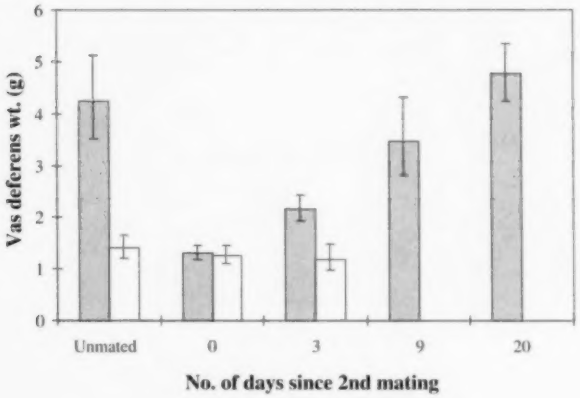
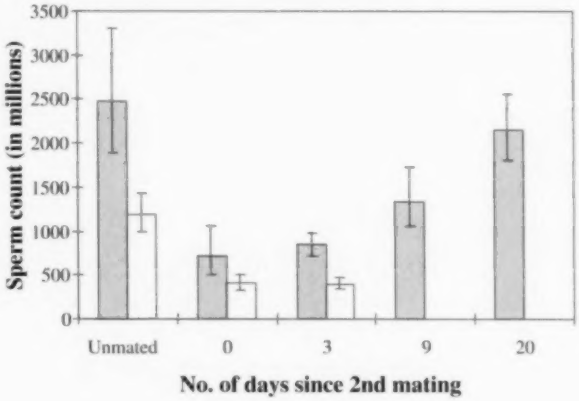


Fig. 3. Sperm counts of small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.



Immediately after mating, large males had a very low mean vas deferens weight (1.31 g) that was significantly lower than that of large males allowed even a 3-day recovery (2.16 g) ($t = 2.07$, $p < 0.04$). The mean vas deferens weight of large males allowed to recover for 3 days was significantly lower than that of large unmated males ($t = 2.52$, $p < 0.02$) or large males allowed to recover for 20 days ($t = 3.14$, $p < 0.004$; Fig. 2). Nine days following mating, large males had a mean vas deferens weight significantly higher than that of males sacrificed immediately after mating ($t = 3.39$, $p < 0.002$) but not significanly different from that of large unmated males and those allowed a 20-day recovery.

Regardless of recovery time, small males consistently had low vas deferens weight that was significantly below that of large males (except large males allowed a 0- or 3-day recovery time following mating). There were no differences in mean vas deferens weight among small unmated males (1.41 g), small males that had just mated (1.27 g), and small males allowed a 3-day recovery (1.19 g).

Sperm counts revealed no significant differences between small unmated males and large unmated males (Fig. 3), despite the weight of the vasa deferentia of small males being only a fraction of that of a large male (Fig. 2). Large males sacrificed immediately after mating and those allowed a 3-day recovery had very low sperm counts (7.2×10^8 and 8.4×10^8 sperm, respectively) that were significantly less than those of large unmated males (2.5×10^9 sperm) ($t = 2.74$, $p < 0.01$ and $t = 2.32$, $p < 0.03$, respectively) and large males allowed a 20-day recovery (2.1×10^9 sperm) ($t = 2.64$, $p < 0.01$ and $t = 2.22$, $p < 0.04$, respectively). Nine days following mating, the sperm counts of large males were intermediate between (and statistically indistinguishable from) those of unmated males and those that had just mated.

Small males allowed a 0- or 3-day recovery time had the lowest sperm counts of all groups (4.1×10^8 and 4×10^8 sperm, respectively); these values were significantly lower than those of small unmated males (1.2×10^9 sperm) ($t = 2.84$, $p < 0.008$ and $t = 2.89$, $p < 0.007$, respectively), despite no significant difference in vas deferens weight (Fig. 3).

Mating history also had an influence on sperm concentration in small males. Small unmated males had a higher mean sperm concentration (1.01×10^9 sperm cells × (vas deferens weight (g))$^{-1}$) than small males evaluated just after mating (2.97×10^8 sperm cells × (vas deferens weight (g))$^{-1}$) ($t = 3.99$, $p < 0.0004$) or those allowed a 3-day recovery (4.25×10^8 cells × (vas deferens weight (g))$^{-1}$) ($t = 3.33$, $p < 0.002$; Fig. 4). No significant differences in sperm concentration were found among large males or large and small males with similar mating histories.

The quantity of reproductive resources delivered by males was estimated by measuring differences in vas deferens weight and sperm counts before and after the experimental matings. Large males experienced an average drop of 2.92 g (70%) in seminal material and 1.78×10^9 (70%) sperm as a result of the two mating events, whereas for small males, seminal fluid decreased by only 0.14 g (10%) and sperm by 7.9×10^8 (70%) as a result of the two mating events.

Reproductive condition of males in the Rhode River

The sex ratio for crabs caught in trawls was highly variable over the course of the reproductive season in both years. The mean ratio of males with a CW over 110 mm (the minimum size at which males were observed paired with females in the field) to pubertal females was ~2:1 in 1996 and ~7:1 in 1997.

There were no differences in mean vas deferens weight among locations in the Rhode River subestuary ($F_{(3,3)} = 2.29$, $p < 0.25$) or among months ($F_{(4,2)} = 3.01$, $p < 0.26$), during the reproductive season. Intact crabs had a higher mean vas deferens weight than crabs missing or regenerating limbs (pooled mean, 1.57 vs. 1.24 g; $F_{(1,244)} = 4.85$, $p < 0.028$). Molt stage B crabs had significantly lower mean

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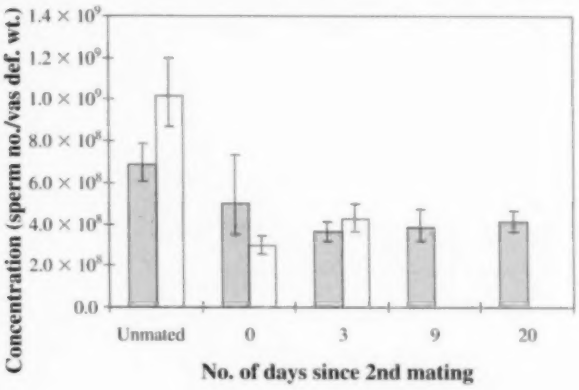
Fig. 4. Sperm concentration (number of sperm/vas deferens weight) for small males (with carapace width (CW) below the legal limit of the fishery (<127 mm); denoted by open bars) and large males (with CW > 140 mm, well above the legal limit of the fishery; denoted by shaded bars) prevented from mating (unmated) and sacrificed 0, 3, 9, and 20 days after two consecutive matings (small males not tested at 9 and 20 days). Means ± standard error of the mean are plotted.



vas deferens weight than molt stage C crabs (0.52 vs. 1.46 g; $t_{244} = 5.05$, $p < 0.0001$). No other differences in vas deferens weight among molt stages were found, although a larger sample size for molt stage A, D, and E crabs would allow the effects of molt stage to be interpreted with more confidence.

At all times during the 1996–1997 mating seasons, at least 50% of the males in the population were scored as "depleted" (had vas deferens weight below that expected had they mated a single time following complete recovery of sperm volume; Figs. 5 and 6). In both years, during July when the mating season begins in earnest, depleted males comprised as much as 64–74% of the male population (Figs. 5*a* and 6*b*). Furthermore, there was a large number of males that even had vas deferens weights much lower than the average value expected had they mated once after fully recovering their sperm volume (i.e., below 1.38 g).

Discussion

A large proportion of the males across all size-classes in the Rhode River had low vas deferens weight. A variety of causes may be responsible. We demonstrated that small males simply have low vas deferens weight. Other males may have gametogenesis and production of seminal fluid precluded by the energetic requirements of molting or limb regeneration. At any given time, 20–30% of crabs in the Rhode River basin are either premolt or post molt (Hines et al. 1987) and 17–25% of crabs are missing and (or) regenerating at least one limb (Smith and Hines 1991). Males with significant limb loss are less competitive for mates than intact males (Smith 1992). For molting or injured males, energetic expenditure on sperm production may not contribute to their reproductive success as much as energy put toward regeneration of chelae or limbs or calcification of their exoskeleton. Another contributing factor may be simply that a high incidence of mating in the population leaves many males depleted.

At first consideration, the absolute sex ratios of 2:1 and 7:1 for the 2 years of this study suggest that there are plenty of males to mate with the female population (without regard to the amount of sperm females receive). However, these are probably very liberal estimates of the "operational sex ratio," a value that provides a more accurate depiction of which males in the population are likely to mate. To estimate this value, we should eliminate or devalue the males that are not likely to be mating (e.g., males that are molt stages A, B, D, and E (which are never observed paired in the field) and those that have lost even one cheliped and are significantly less able to defend females from intact competitors; Smith 1992). Competitive interactions between males can also influence the operational sex ratio. Laboratory experiments revealed that large males have better pairing and mate-stealing success than small males (Jivoff 1997*a*, 1997*b*). The mating history of males is also a determinant of pairing success (Jivoff 1997*a*, 1997*b*). Males that have recently mated (and therefore have low vas deferens weight and sperm count; see Results) are, in fact, better competitors for mates than males without recent experience (Kendall and Wolcott 1999). Given these considerations, the operational sex ratio will always be less than the absolute ratio of adult males to pubertal females, and an estimate of 1:1 may be more appropriate for the Rhode River system.

There were no significant differences in mean vas deferens weight among locations or months over the reproductive season in the Rhode River. However, these are not the most informative comparisons for examining spatial and temporal trends in male reproductive condition. Females do not mate with the "*average*" male crab; females receive sperm from *individual* males in the population. Examining mean reproductive condition instead of reproductive condition of individuals can be misleading. For example, in a population, the average amount of stored sperm per male may be high but, if individual males that have very low sperm volume mate with some percentage of the females, those females may be sperm limited. Conversely, the average level of stored sperm per male in a population may be very low but, if the few males in the population with a large volume of stored sperm are competitive dominants, they may do most of the mating and provide their mates with ample sperm. Therefore, rather than examining mean vas deferens weight over some time interval or location, we suggest that scatter plots of vas deferens weight versus crab size yield a more meaningful biological perspective on the reproductive condition of the male population.

We observed a large number of males in the population with a vas deferens weight much lower than that expected had they just mated a single time after fully recovering their sperm volume. This suggests that these males lack a full complement of sperm and (or) seminal fluid between consecutive mating events. In fact, many males in the field were observed to have vas deferens weights much lower than even the most depleted groups observed in the laboratory study. Females mated with these males would receive particularly low quantities of sperm and seminal fluid.

Following mating, large males recover most of their seminal resources (sperm and seminal fluid) in less than 9 days.

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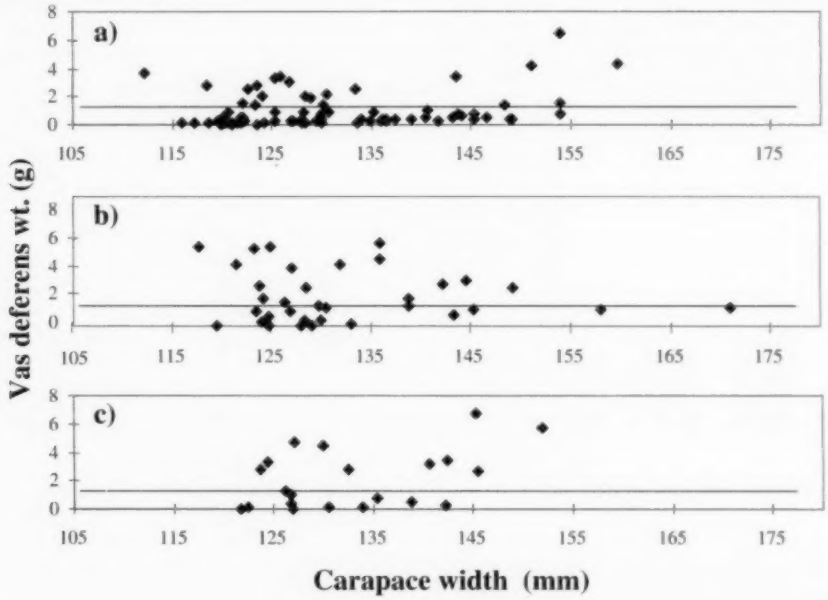
Fig. 5. Scatter plots of vas deferens weight (g) versus carapace width (mm) for pooled trawls during July–September (*a–c*) of the 1996 reproductive season. The horizontal line in each plot denotes the vas deferens weight of a crab allowed full recovery of sperm resources and then mated a single time.



Vas deferens weight is recovered very quickly; as few as 9 days were required for large males to have a vas deferens weight significantly higher than that of males which had just mated and statistically indistinguishable from the vas deferens weight of fully recovered males. However, the recovery of sperm number probably takes longer and is apparently still underway at 9 days following copulation; at this point, large males have levels of sperm that are intermediate between just-mated males and fully recovered males and overlap statistically with both. Despite these small differences in the recovery rate of sperm number and seminal fluid for large males, the experimental samples demonstrate that, for large males, complete recovery of all seminal material is attained between 9 and 20 days following mating. Small males require more than 3 days to recover sperm number (vas deferens weight does not vary), although the time required for complete recovery cannot be determined from this study (we did not follow a group of small males for a longer recovery period, because vas deferens weight levels had not dropped much following mating). Since males typically guard females for 2–5 days after mating and for ~5 days prior to mating (these values are estimated from field observations and laboratory experiments, respectively; Jivoff 1995), the sum of these times suggests that large males that mate repeatedly would have sufficient time to replenish most of their number of spermatozoa and at least some of their seminal fluid (vas deferens weight) between consecutive matings. However, large males are being selectively removed from the population by the fishery. The fisheries mortality (F) on age 1+ is estimated to be as high as 0.8–0.9 in some years (Sauls et al. 1995). Those males that remain will spend less time guarding mates as the sex ratio declines (Jivoff 1997*a*), which will reduce the time available for recovery of reproductive resources between consecutive matings. Furthermore, small males that escape the fishery are left to service a large portion of the female population. Field observations in the Rhode River revealed that sublegal males are paired with approximately one-third of receptive females (Jivoff 1995, 1997*b*). Males below the legal size limit of the fishery generally have available only a fraction of the seminal material of large males. Females mated with males of below 127 mm CW probably received smaller quantities of seminal fluid and, possibly, fewer sperm than if they had mated with a large recovered male.

We were also able to infer the amount of seminal material that males delivered to females by comparing vas deferens weight and sperm number observed in males before and after mating. In our experiments, both large and small males delivered approximately 70% of their stored sperm, although the difference in absolute numbers was bigger for large males. In fact, large males delivered 2.25 times as much sperm and 21 times as much seminal fluid as small males. Interestingly, small males experienced essentially no change in vas deferens weight following mating, whereas large males lost nearly 70% of their premating vas deferens weight, indicating that small males deliver scant amounts of accessory seminal fluid with their sperm.

The delivery of male reproductive resources is affected by the interaction of male size and mating history. Additionally, levels of reproductive resources are characterized by high variability, both in the experimental crabs and in the field population. While large fully recovered males appear to be capable of delivering much greater quantities of seminal fluid and sperm than small males, the levels of reproductive

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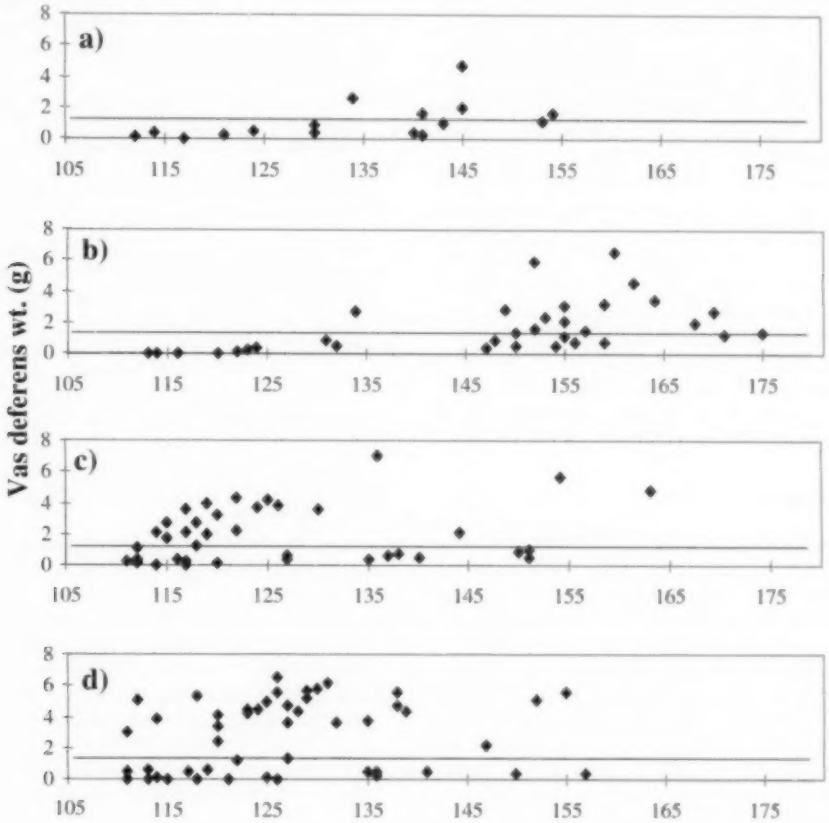
Fig. 6. Scatter plots of vas deferens weight (g) versus carapace width (mm) for pooled trawls during June–September (*a–d*) of the 1997 reproductive season. The horizontal line in each plot denotes the vas deferens weight of a crab allowed full recovery of sperm resources and then mated a single time.



resources for many large males in nature may be similar to those of small males, especially if the large male has been mating recently.

Females mating with large depleted males or small males of any reproductive history receive small quantities of seminal fluid. The long-term consequences of receiving less seminal fluid are unclear. Seminal fluid is not only the medium in which sperm are transferred but also has been suggested to influence reproductive success by serving as a source of sperm nutrition during storage (Subramoniam 1993), as an antibacterial agent protecting the female reproductive tract (Jayasaker and Subramoniam 1999), and as a "sperm plug" that may both prevent deposited sperm from leaking out of a female's seminal receptacles and deny access to a female's reproductive tract to subsequent males (Ryan 1964; Hartnoll 1969). Males delivering reduced quantities of seminal fluid but normal numbers of spermatocytes may experience reduced reproductive success due to sperm loss from inadequate nutrition, bacterial infection, or simple leakage from a female's reproductive tract.

It should be noted that the dual role of the "sperm plug" might have both positive and negative influences on the possibility of sperm limitation. Since smaller males have less seminal fluid, their sperm plugs may be less effective. While this may result in some sperm leakage (making sperm limitation more likely), it could increase the potential for a female to receive sperm from two males because of an ineffective plug; this would reduce her likelihood of being sperm limited.

We have documented high incidences of males with very reduced seminal resources in a natural population and demonstrated that stored sperm and seminal fluid become depleted as a result of mating and remain so for some days. These results confirm the importance of two potential mechanisms by which the reproductive potential of the blue crab mating system could be limited by reproductive resources contributed by males (sperm and (or) seminal fluid): (1) a reduction in the male:female operational sex ratio and (2) a high incidence of mating by small or frequently mating males with low vas deferens weight (for large males, frequent mating is probably not a source of reduced fertilization success, if mate-guarding times observed in the laboratory accurately reflect those in the field). To understand the impact of these mechanisms on the reproductive output of females and definitively determine if sperm limita-

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tion is occurring, it remains to (*i*) determine the amount of sperm and seminal fluid received by females in the field, (*ii*) examine the influences of male size and mating history on the amount of sperm and seminal fluid actually transferred to females, (*iii*) quantify the relationship between sperm number, seminal fluid volume, and fertilization success, (*iv*) identify additional influences on operational sex ratio and their effects on the reproductive potential of the population, and (*v*) identify the role(s) of seminal fluid in the blue crab mating system.

Proper management of the fishery must incorporate all aspects of the stock–recruit relationship. Often, management strategies consider only mature females in this regard (Jones et al. 1990; Sauls et al. 1995; Prager 1996). Blue crabs and other crustacean species appear to be susceptible to fishery-induced sperm limitation (Smith and Jamieson 1991; Paul and Paul 1992; Sainte-Marie and Lovrich 1994). Our study suggests that we must consider the attributes of the male contribution to reproductive success (i.e., sperm and accessory seminal fluid), to obtain an accurate assessment of reproductive potential at the population level in the blue crab.

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A semiparametric model to examine stock–recruitment relationships incorporating environmental data

D.G. Chen and J.R. Irvine

Abstract: A novel semiparametric model that can incorporate environmental and fishery data is developed to analyze stock–recruitment relationships. Unlike traditional stock–recruitment models that assume a log-linear relationship between recruitment and environmental and fishery variables, the new model uses a nonparametric smoothing algorithm, which helps quantify the underlying empirical relationships and enables more accurate parameter estimates. Bootstrap resampling is used to evaluate uncertainties in the model parameters. Distribution plots can be produced for stock–recruitment productivity and capacity parameters. This approach is applied to southeast Alaska pink salmon (*Oncorhynchus gorbuscha*) with sea surface temperature as the environmental variable and West Coast Vancouver Island herring (*Clupea harengus*) with sea surface temperature and hake biomass as two environmental variables. Results from diagnostic tests indicate that our model performed better than the traditional Ricker model and a Ricker model that was extended to include environmental effects.

Résumé : On trouvera ici un nouveau modèle semiparamétrique capable d'incorporer les données environnementales et halieutiques dans l'analyse des relations entre le stock et le recrutement. À l'opposé des modèles traditionnels de stock–recrutement qui posent au départ l'existence d'une relation log-linéaire entre le recrutement et les variables environnementales et halieutiques, le nouveau modèle utilise un algorithme non-paramétrique de lissage qui aide à quantifier les relations empiriques sous-jacentes et permet une estimation plus précise des paramètres. Le ré-échantillonnage par bootstrap sert à évaluer les incertitudes dans les paramètres du modèle. Des diagrammes de dispersion peuvent être générés pour les paramètres de productivité et de capacité du modèle stock–recrutement. Le modèle est appliqué à une population de Saumons roses du sud-est de l'Alaska (*Oncorhynchus gorbuscha*) avec la température de surface de la mer comme variable environnementale, ainsi qu'à une population de harengs de la côte occidentale de l'Île de Vancouver (*Clupea harengus*) où la température de surface de la mer et la biomasse des merlus sont les variables environnementales. Des tests diagnostics révèlent que le nouveau modèle fonctionne mieux que le modèle traditionnel de Ricker et qu'un modèle de Ricker modifié de façon à inclure les effets de l'environnement.

[Traduit par la Rédaction]

Introduction

Stock–recruitment (S–R) analysis is often the first step when evaluating and implementing fishery policies designed to optimize spawner numbers and exploitation rates. The analysis typically begins with the assumption of a functional relationship, denoted by $F(\cdot)$, between spawners and recruits:

$$(1) \qquad R_t = F(S_t,\theta)$$

where R_t and S_t are the recruits and spawners, respectively, at year t, $t = 1,\ldots,n$. θ is a vector of parameters associated with this relationship and usually is associated with the fishery management policy.

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The functional form proposed by Ricker (1975) is the most commonly used model:

$$(2) \qquad R_t = S_t e^{a-bS_t}$$

where a and b are parameters for measures of productivity and capacity, respectively. These parameters can be estimated using standard linear regression analysis:

$$(3) \qquad \log(R_t/S_t) = a - bS_t.$$

Associated fishery management parameters (i.e., optimal stock size S_{MSY} and harvest rate u_{MSY} at maximum sustainable yield (MSY)) can then be calculated from this model as described in Hilborn and Walters (1992) and Quinn and Deriso (1999).

Although recruitment measured as the number of fish alive at spawning is determined to a large degree by the number of spawners in the parental generation, natural environmental factors (e.g., water temperature and salinity, etc.) and human-induced fishery interventions can also play a significant role. Environmental changes are often correlated with survival and growth of young fish (Ware and McFarlane 1995; Chen and Ware 1999; Chen et al. 2000). Changes in harvest rates resulting in altered recruitment

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(e.g., Cass and Riddell 1999; Ryall et al. 1999) and enhancement facilities that increase the numbers of fish available to be caught (e.g., Lichatowich 1999) are examples of fishery interventions.

Therefore the S–R relationship in eq. 1 can be expressed as

$$(4) \qquad R_t = F(S_t, \mathbf{Z}_t, \theta)$$

where $\mathbf{Z}_t$ is a variable vector associated with environmental effects and (or) fishery interventions. In this paper, our examples only incorporate environmental data, although the model could use fishery intervention data equally well.

Others have incorporated environmental information into their S–R analysis. Ricker (1958) considered the effects of environmental variability on stock and recruitment models. Tang et al. (1989) modified the Ricker S–R model to account for environmentally induced variation in recruitment of prawns. Stocker et al. (1985) fit a Ricker S–R model to herring (*Clupea harengus*) data from the Strait of Georgia, British Columbia, incorporating various environmental factors including sea surface temperature and Fraser River discharge. Chen and Ware (1999) included sea surface temperature, salinities, and hake (*Merluccius productus*) biomass in a Ricker S–R model. Planque and Fredou (1999) incorporated temperature data in a S–R analysis of Atlantic cod (*Gadus morhua*). From these works, one can extend the traditional Ricker model (eq. 2) to incorporate environmental effects and fishery interventions:

$$(5) \qquad R_t = S_t e^{a - bS_t + c_1 Z_{1t} + \ldots + c_p Z_{pt}}$$

where $Z_{1t}, \ldots, Z_{pt}$ are environmental variables and (or) fishery interventions. We will refer to eq. 5 as the extended Ricker S–R model. The application of this model is based on a log transformation of eq. 5:

$$(6) \qquad Y_t = X_t\beta + \mathbf{Z}_t\gamma$$

where $Y_t = \log(R_t/S_t)$, $X_t = (1, S_t)$, $\beta = (a, -b)'$, $\gamma = (c_1, \ldots, c_p)'$, and $\mathbf{Z}_t = (Z_{1t}, \ldots, Z_{pt})$.

Therefore, environmental effects and fishery interventions are incorporated into the S–R model in a log-linear form. Parameters from the extended Ricker model (eq. 6) can be estimated using multiple linear regression.

Arguments can arise regarding the functional form of variable vector $\mathbf{Z}_t$ because, in reality, the relationship between recruitment and $\mathbf{Z}_t$ may not be log-linear as described in eq. 5. Such a relationship, although it exists, is difficult to describe with a simple model. One option is to relate R_t and $\mathbf{Z}_t$ by a power law as $R_t = \mathbf{Z}_t^c S_t \exp(a - bS_t)$, with $\mathbf{Z}_t$ interpreted as survival rate and c is a parameter. Using this formulation, $\log(R_t)$ and $\log(\mathbf{Z}_t)$ are linearly related. In real fishery applications, Stocker et al. (1985) found the relationship between $\log(R/S)$ and sea surface temperature (SST) to be dome-shaped for Strait of Georgia herring. Fargo and McKinnell (1989) also found a dome-shaped relationship between year-class production of rock sole (*Lepidopsetta bilineata*) and SST. Jacobson and MacCall (1995) imply nonlinear behavior between the log reproductive success and the mean SST for pacific sardines (*Sardinops sagax*).

In this paper, we relax this log-linear assumption and develop a nonparametric functional form for the auxiliary variable vector $\mathbf{Z}_t$. We retain the linearized S–R model to comply with traditional S–R analysis so that fisheries managers can assess stock status from S–R relationships. This leads to the following semiparametric model that describes a stock–recruitment relationship with environmental effects and fishery interventions:

$$(7) \qquad Y_t = X_t\beta + f(\mathbf{Z}_t) + \varepsilon_t$$

where $Y_t = \log(R_t/S_t)$, $X_t = (1, S_t)$, $\beta = (a, -b)'$, and $\varepsilon_t \sim (0, \sigma^2)$. $f(\mathbf{Z}_t)$ denotes the general functional form of the p-variable vector $\mathbf{Z}_t$. To simplify the discussion, if $\mathbf{Z}_t = (Z_{1t}, \ldots, Z_{pt})$, we take $f(\mathbf{Z}_t) = f_1(Z_{1t}) + \ldots + f_i(Z_{pt})$ with an additive form. Equation 7, an extension of eq. 5, is in fact an additive model and includes two components. The first component is the parametric term $X\beta$, which is equivalent to the left side of the traditional Ricker S–R model (eq. 3). The second is the nonparametric term $f(\mathbf{Z}_t)$, which can only be estimated by a nonparametric approach, such as nonparametric smoothing. This semiparametric model (eq. 7) is hereafter referred to as the semiparametric S–R model (SPSRM).

The purpose of this paper is to describe our new SPSRM and the approach that we advocate portraying the distribution around various stock–recruitment parameters. We compare the utility of SPSRM with Ricker models for southeast Alaska (SEAK) pink salmon (*Oncorhynchus gorbuscha*) with SST as the environmental variable and for West Coast of Vancouver Island (WCVI) herring with SST and hake biomass as two environmental variables.

Statistical estimation for SPSRM

The SPSRM includes a parametric term $X\beta$ with a parameter vector β and a nonparametric term $f(Z)$. If there are no environmental effects or fishery intervention variables, the SPSRM can be reduced to the Ricker model (eq. 3), and one can use regression analysis to estimate the S–R parameter vector β. Otherwise, one can apply nonparametric smoothing techniques to the SPSRM to estimate the parameter vector β along with the $f(Z)$. The most commonly used approach is the back-fitting algorithm in Hastie and Tibshirani (1990). This algorithm, which provides a point estimate of β, has been implemented in many popular statistical software packages, such as S-PLUS and Genstat. Unfortunately, the algorithm that gives the standard errors for the estimated $\hat{\beta}$ is not applicable as it involves fitting (nonparametric smoothing) n models. As well, the distribution for $\hat{\beta}$ is based mostly on the central limit theorem (Robinson 1988; Andrews 1991; Chamberlain 1992), which requires a large sample size n. This is not appropriate for S–R analysis because n is rarely large.

In this paper, we use a bootstrapping approach to obtain sampling distributions for the S–R parameter vector β, which allows us to make statistical inferences based on the sampling distribution. When one wishes to provide additional estimates of management parameters, such as MSY spawners or exploitation rates, this bootstrapping approach can be used to generate the distributions for these management parameters so that one can provide not only a point estimate, but also precision estimates associated with each point estimate.

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Parameter estimation

We begin with a simple case of $p = 1$ in the SPSRM, i.e., estimating a single nonparametric term, f_1. This simple case will be used later in the paper when we incorporate SST as an environmental variable for SEAK pink salmon.

Suppose that f_1 is fitted with a smoother $\mathbf{M}_1$, such as smoothing spline, locally weighted regression (loess), or kernel-type smoothing. It is known (Green and Silverman 1994; Hastie and Tibshirani 1990) that

$$(8) \qquad \hat{\boldsymbol{\beta}} = [\mathbf{X}'(\mathbf{I} - \mathbf{M}_1)\mathbf{X}]^{-1}\mathbf{X}'(\mathbf{I} - \mathbf{M}_1)\mathbf{Y}$$

provided that $\mathbf{X}'(\mathbf{I}-\mathbf{M}_1)\mathbf{X}$ is invertible. In eq. 8,

$$\mathbf{X} = \begin{pmatrix} X_1 \\ \vdots \\ X_n \end{pmatrix} = \begin{pmatrix} 1 & S_1 \\ \vdots & \vdots \\ 1 & S_n \end{pmatrix},$$

which is a $n \times 2$ matrix with all the elements in the first column being 1 and the second column being the spawner time series from year 1 to the last year n;

$$\mathbf{Y} = \begin{pmatrix} \ln\left(\dfrac{R_1}{S_1}\right) \\ \vdots \\ \ln\left(\dfrac{R_n}{S_n}\right) \end{pmatrix},$$

which is a n-dimensional vector for the logged recruitment per spawner, and $\mathbf{I}$ is the $n \times n$ identity matrix.

A natural extension is to consider the situation of $p > 1$, i.e., more auxiliary environmental variables, which is what we do in our WCVI herring example when we incorporate SST and hake biomass. For this type of situation, Durban et al. (1999) proposed the following estimator for any number of nonparametric terms under certain weak assumptions:

$$(9) \qquad \hat{\boldsymbol{\beta}} = [\mathbf{X}'(\mathbf{I} - \mathbf{M}_p)\mathbf{X}]^{-1}\mathbf{X}'(\mathbf{I} - \mathbf{M}_p)\mathbf{Y} = A\mathbf{Y}$$

where the matrix $\mathbf{M}_p$ generalized from S_1 in eq. 8 is a centered hat matrix because it can be expressed as

$$(10) \qquad \mathbf{M}_p = \mathbf{M}_p^* - \frac{\mathbf{1}\mathbf{1}'}{n}$$

where $\mathbf{1}$ is a $n \times 1$ vector with all elements being 1 and $\mathbf{M}_p^*$ is a smoothing matrix for the model $y = a + f_1 + \ldots + f_p + \varepsilon$. Hastie and Tibshirani (1987) proposed a method to find $\mathbf{M}_p$ by fitting its values to the following n models: $e_j = \alpha + f_1 + \ldots + f_p + \varepsilon$, where e_j is the jth column of the identity matrix $\mathbf{I}_n$. The fitted values from these n models constitute matrix $\mathbf{M}_p^*$ and hence $\mathbf{M}_p$ via eq. 10. This scheme to fit n models can be computationally difficult, especially when n is large.

Bootstrapping scheme

Although the back-fitting approach can be used to estimate the S–R parameter vector $\boldsymbol{\beta}$, the procedures to estimate the standard errors of $\hat{\boldsymbol{\beta}}$ are not well-established when sample sizes are small. To ensure that a proper statistical inference for $\boldsymbol{\beta}$ was made, we used bootstrap resampling to obtain the sampling distribution for $\boldsymbol{\beta}$ (Efron and Tibshirani 1993; Shao and Tu 1995; Davison and Hinkley 1997).

In general, we can treat the input variables (X_t, Z_t) to SPSRM as either deterministic or random variables. In the case of deterministic variables, the residuals ε_t ($t = 1$ to n) are assumed to be identically and independently distributed (i.i.d.) with a zero mean and a constant variance of σ^2. In the case of random variables, data (Y_t, X_t, Z_t) are assumed to be i.i.d. with $E(\varepsilon_t|X_t, Z_t) = 0$. The above assumptions correspond to two different types of settings for bootstrapp resamplings (see Tibshirani 1994). One setting treats the predictors as fixed based on the deterministic variables (X_t, Z_t) with the model residuals $\varepsilon_t = y_t - \hat{y}_t$ as the sampling units, where $\hat{y}_t$ is the estimate from the model (i.e., bootstrap residuals). The other setting treats each data point (Y_t, X_t, Z_t) as a sampling unit (i.e., bootstrap pairing). Wu (1986) reviewed these two bootstrap resampling methods and concluded that some bias would result from bootstrap pairings. To avoid bias and because of the plausible existence of autocorrelation within the S–R data, we advocate the use of bootstrap residuals as a resampling strategy to the proposed sampling approach as in the following steps. (*i*) Fit the SPSRM to the observed data (Y_t, X_t, Z_t ($t = 1$ to n)) and check assumptions of the independence and homogeneity of the residuals $\{\varepsilon_t\}_{t=1}^n$. If assumptions are violated, go back to the SPSRM with suitable transformation to the observed data. When assumptions are accepted, (*ii*) resample with replacement n data points $\{\varepsilon_t\}_{t=1}^n$ from the residuals in step *i* and define the bootstrap data (X_t, Z_t, Y_t^*), where $Y_t^* = X_t\hat{\boldsymbol{\beta}} + \hat{f}(Z_t)$; (*iii*) fit the SPSRM for the bootstrap data from step *ii*; (*iv*) output the estimated S–R parameter vector $\boldsymbol{\beta}$; and (*v*) repeat steps *ii* to *iv* N times (with $N = 1000$ as a suggested number of repeats).

The above procedure will yield a sample for the S–R parameter vector as $\boldsymbol{\beta}_1, \ldots, \boldsymbol{\beta}_N$. This sample can be used to construct a sampling distribution for the S–R parameters a and b. Sampling distributions can be obtained for fishery management parameters, such as MSY spawner, S_{MSY}, and MSY exploitation rate, u_{MSY}, etc., using formulae in Hilborn (1985) or Hilborn and Walters (1992).

Data applications

SEAK pink salmon S–R data

Data description and preliminary analysis

S–R data for SEAK pink salmon from 1960 to 1989 (Fig. 1*a*) and corresponding environmental data are taken from Quinn and Deriso (1999, pages 104–123). To examine S–R, Quinn and Deriso explored eight models: (1) constant recruitment model defined as $\log(R) = a + \varepsilon$; (2) density independent model defined as $\log(R) = a + \log(S) + \varepsilon$; (3) Beverton–Holt model; (4) Ricker model; (5) Cushing model; (6) Deriso–Schnute model; (7) Shepherd model; and (8) Gamma model. They found that while the constant log-recruitment model was the simplest, the density-independent model fit the data better and was statistically significant. They concluded that recruitment increases with spawning levels. For the remaining six S–R models, the estimated density-dependent parameters were not statistically signifi-

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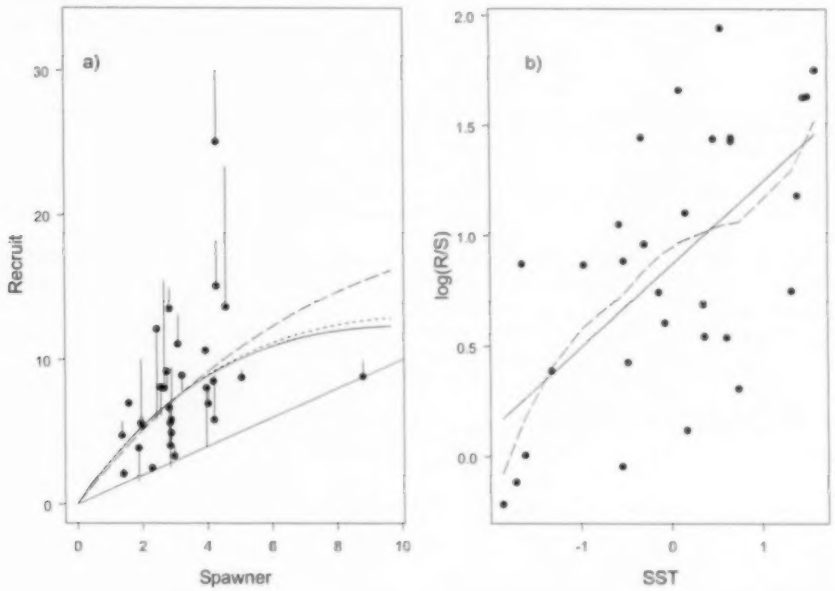
Fig. 1. (*a*) The stock–recruitment (S–R) relationship for southeast Alaska (SEAK) pink salmon from Quinn and Deriso (1999) in units of 1000 fish. The lines from the top to the bottom are the Ricker fit (long broken curve), extended Ricker fit (short broken curve), and the semiparametric stock–recruitment model (solid curve); the straight line is the replacement line. The dots (●) at the end of all vertical segments are the recruits adjusted from the semiparametric stock–recruitment model (SPSRM in eq. 13) with the other end the observed recruitment values. (*b*) The relationship between sea surface temperature (SST, standardized with mean 0 and standard deviation 1) and log(*R*/*S*). Dots (●) are the observed values, the straight line is the linear regression fit, and the broken line resulted from the SPSRM (eq. 7).



cantly different from zero with correlation coefficients close to 1.

To explain some of the variation in recruitment, Quinn and Deriso (1999) introduced the average annual SST off Sitka, Alaska, into their analysis. They showed that the extended Ricker S–R model, generalized to include a SST effect, was statistically significant with the best outcomes based on the model residual sum of squares (SSE) and R^2 values. Although the density-dependent parameter b was still insignificant, it was less so than that produced by the Ricker model (eq. 3) without a SST effect.

Results from SPSRM

Because SST is the only auxiliary environmental variable, the SPSRM is

$$(11) \quad Y_t = X_t\beta + F(Z_{1t}) + \varepsilon_t$$

where Z_1 = SST. The estimation of $f(Z_1)$ is based on nonparametric smoothing of the observed data. This is accomplished by the cubic smoothing spline (CSS) method, which is used to estimate the function f that minimizes the penalized residual sum of squares (PRSS) given by

$$(12) \quad \text{PRSS} = \sum_{t=1}^{n} [Y_t - f(Z_t)]^2 + \lambda \int [f''(Z)]^2 dZ$$

where λ is the smoothing parameter which specifies the degrees of freedom (df) for the smoother and also the weight for the penalty to the sum of squares in eq. 12.

The procedure described in the Parameter estimation section was followed to estimate the stock parameters (a and b) along with the nonparametric function $f(Z_1)$ (Table 1). To compare the outcomes from SPSRM with those from linear models, we also fit the same data against the Ricker model (eq. 3) as $\log(R/S) = a - bS$ and the extended Ricker model (eq. 5) as $\log(R/S) = a - bS + c_1\text{SST}$. Classical regression analysis indicated that the Ricker model was not statistically significant ($p = 0.49$, F test). Including SST as an environmental effect made the extended Ricker model (eq. 5) significant. The SPSRM was also statistically significant, based on a residual deviance of 5.63 and a smoothing df of 24, which yielded a probability of 0.99 for χ^2 goodness of fit. SPSRM exhibited the lowest SSE and highest R^2 among the three models. In Fig. 1*a*, we illustrate the S–R relationships derived from the Ricker model (eq. 3), the extended Ricker model (eq. 5), and the SPSRM (eq. 7). In this plot, we also illustrate the adjusted recruits from SST from the SPSRM as defined by

$$(13) \quad \tilde{R} = R \times \exp(-\widehat{f(Z)})$$

where $\widehat{f(Z)}$ is the estimated smoothing function from SPSRM with Z = SST. A substantial adjustment from the environmental effect (SST) was achieved from this SPSRM (Fig. 1*a*). Also as seen from Fig. 1*b*, the smoothed relationship between the SST and the stock productivity, log(*R*/*S*), is suggestive of potential nonlinearity although the uncertainty in the relationship is large.

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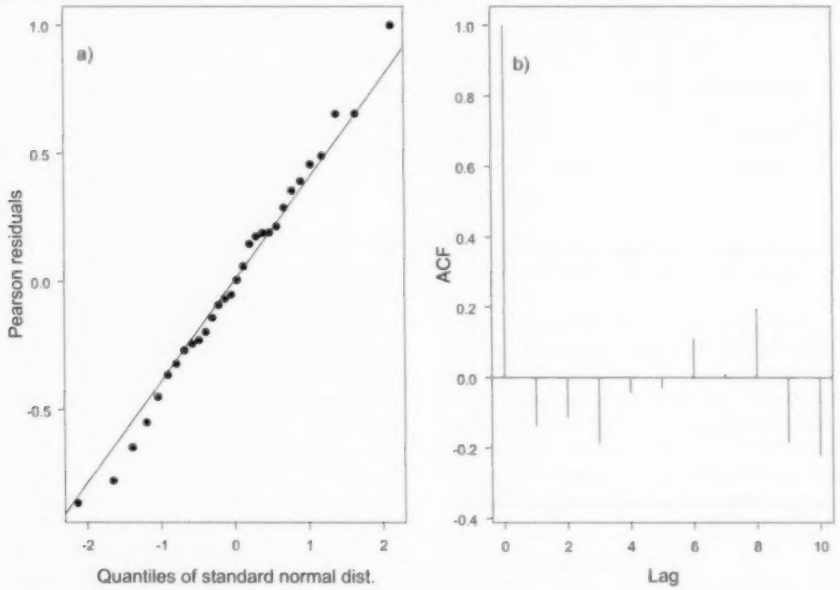
Table 1. Summary of fit for various models.

	SEAK pink salmon			WCVI herring		
Model	Ricker	Extended	SPSRM	Ricker	Extended	SPSRM
a	1.05 (0.28) S	1.16 (0.27) S	1.18 (0.21) S	0.37 (0.27) NS	0.51 (0.25) NS	0.75 (0.16) S
b	5.52×10^{-5}	9.03×10^{-5}	9.64×10^{-5}	2.54×10^{-2}	3.01×10^{-2}	3.85×10^{-2}
	(7.89×10^{-5}) NS	(6.18×10^{-5}) NS	(6.02×10^{-5}) NS	(7.11×10^{-3}) S	(6.80×10^{-3}) S	(4.21×10^{-3}) S
c_1	NA	0.39 (0.09) S	NA	NA	–0.46 (0.16) S	NA
c_2	NA	NA	NA	NA	–0.18 (0.16) NS	NA
SSE	10.45	6.06	5.63	47.46	37.75	15.03
R^2	0.02	0.43	0.46	0.24	0.40	0.76
P (model fit)	NS	S	S	S	S	S

Note: "Ricker" denotes the traditional Ricker model (eq. 3) and "Extended" denotes the extended Ricker model (eq. 5). For parameters a, b, c_1, and c_2, the value inside the parentheses is the associated standard error. S or NS for parameters a, b, c_1, and c_2 indicates that the parameter is statistically significant (S) or statistically non-significant (NS) at the 95% confidence level. SSE denotes the sum of squares for the residuals. The last row denotes the model significance based on the F-statistic for the Ricker and extended Ricker models and χ^2 test for SPSRM at the 95% confidence level. In the table, SEAK = southeast Alaska, WCVI = West Coast Vancouver Island, SPSRM = semiparametric stock–recruitment model, NA = not available.

Fig. 2. (*a*) The quantile–quantile plot for the residuals from the semiparametric stock–recruitment model (SPSRM (eq. 7)) and the standard normal distribution. The points are approximately on a straight line, indicating that the residuals are normally distributed. (*b*) The time series autocorrelation function (ACF) plot for the residuals to check for autocorrelation. Because all autocorrelation coefficients are within the 95% confidence bands (dotted lines), there appears to be no autocorrelation for the residuals.



Examination of the residual diagnostics for the SPSRM (eq. 11) (Fig. 2*a*) indicates that the residuals are normally distributed, which was confirmed by a Kolmogorov–Smirnov test ($p > 0.5$). The autocorrelation function plot for the residuals (Fig. 2*b*) demonstrated that there is no autocorrelation among the residuals. The residual plot between the residuals and the predicted values shows the homogeneity. Therefore the residuals are i.i.d. as normal.

In this situation, it is legitimate to use the bootstrapping approach described in the Bootstrapping scheme section. We ran the bootstrapping approach 1000 times; the resultant resampling distributions for Ricker parameters a and b are shown in Fig. 3. For the two bootstrapping resampling distributions in Fig. 3, the 95% confidence intervals are (0.79, 1.61) and (-2.28×10^{-3}, 6.02×10^{-3}), respectively, for parameters a and b, which yield similar conclusions for the statistical significance to that obtained with the Ricker model and the extended Ricker model (Table 1). However, the standard errors for both parameters are smaller than for the Ricker models. In addition, the application of bootstrapping for the SPSRM does not require any distribution assumption.

WCVI herring data

Data description and preliminary analysis

A long-term study of WCVI herring found that the key variables affecting the biomass of 3-year-old herring recruits (in year t) were the biomass of spawners (i.e., parents) in the year when the recruits were born, i.e., in year $t - 3$; the mean annual SST in year $t - 3$; and the biomass of hake (an important predator) in year $t - 3$ (Chen and Ware 1999). Thus,

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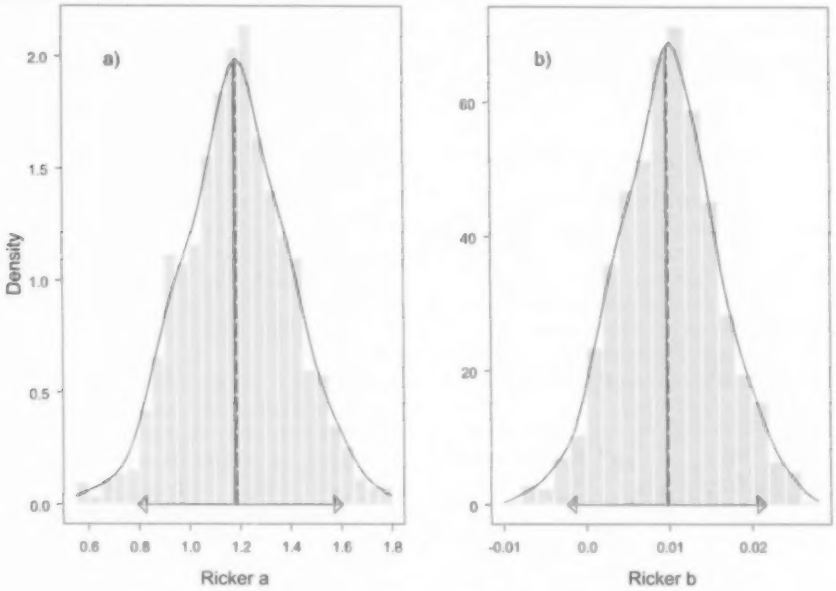
Fig. 3. Bootstrapping distributions for the stock–recruitment parameters a and b from southeast Alaska pink salmon (1000 runs) with parameter b rescaled to 100 times. For both plots, the curved line is the kernel density smoothing estimate. The sample mean and the observed value are marked as vertical broken and solid lines, respectively. The horizontal lines with open arrows on both ends indicate the sample 95% confidence intervals.



we select the herring spawning biomass, SST, and hake biomass as the significant variables for the analysis of herring recruitment.

Results from SPSRM

Because SST and the hake biomass are the auxiliary environmental variables in this situation, the SPSRM (eq. 7) can be formulated as

$$(14) \quad Y_t = X_t\beta + f_1(Z_{1t}) + f_2(Z_{2t}) + \varepsilon_t$$

where Z_1 = SST and Z_2 = hake biomass. The estimation of $f_1(Z_1)$ and $f_2(Z_2)$ is based on nonparametric smoothing of the observed data and we use the CSS method to estimate these functions from the minimization of the PRSS.

Following the procedure described earlier in the Parameter estimation section, the S–R parameters (a and b) were estimated along with the nonparameteric functions $f(Z_1)$ and $f(Z_2)$ (Table 1). Also we fit the same data with the Ricker model (eq. 3) and the extended Ricker model (eq. 5) as $\log(R/S) = a - bS + c_1\text{SST} + c_2(\text{hake biomass})$. Each model was significant (Table 1). However, because the SPSRM had the lowest SSE and the highest R^2, we concluded that it was superior.

Figure 4*a* graphically illustrates the S–R relationship, the three model fits, and the adjustments to the S–R relationship from the SPSRM. It can be seen that the adjustments are considerable.

Figures 4*b* and 4*c* are the estimated smoothing functions that resulted from SPSRM. Both figures showed that a nonlinear relationship is more reasonable than the simple linear regression.

The residual diagnostics for SPSRM (eq. 14) have shown that there is no departure from the i.i.d. normal distribution; therefore, we ran the bootstrapping approach described in the Bootstrapping scheme section 1000 times. The resultant resampling distributions for Ricker parameters a and b are shown in Fig. 5. For the two bootstrapping resampling distributions in Fig. 5, the 95% sample confidence intervals are (0.42, 1.04) and (2.94×10^{-2}, 4.61×10^{-2}), respectively, for parameters a and b. The sample standard errors are smaller than produced using the Ricker model and the extended Ricker model. In addition, the application of bootstrapping for the SPSRM does not require any distribution assumption.

Discussion

The semiparametric approach developed in this paper for stock–recruitment analysis is based on extensions of the traditional Ricker model and extended Ricker model. In this paper, we incorporated data from environmental effects in our new model (SPSRM); other types of information, e.g., fishery intervention data, could also have been used. Unlike traditional linear regression models, SPSRM adopts a nonparametric smoothing algorithm that helps quantify underlying empirical relationships. This enables more accurate parameter estimates and consequently better advice for fisheries management.

In their seminal treatise on quantitative stock assessment, Hilborn and Walters (1992) caution against incorporating environmental variables in stock–recruitment analyses. We share their concern that a model with little or no predictive ability may be produced if spurious correlations are incorporated into it. We feel that it was appropriate to use the environmental variable SST in our examples because it is correlated

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Fig. 4. (*a*) The stock–recruitment relationship for West Coast Vancouver Island herring. The lines from the top to the bottom are the Ricker fit (long broken curve), extended Ricker fit (short broken curve), and the semiparametric stock–recruitment model (solid curve); the straight line is the replacement line. The dots (●) at the end of all vertical segments are the recruits adjusted from the semiparametric stock–recruitment model (SPSRM in eq. 13) with the other end being the observed recruitment values. (*b*) The relationship between sea surface temperature (SST, standardized with mean 0 and standard deviation 1) and log(*R*/*S*). Dots (●) are the observed values, the straight line is the linear regression fit, and the broken line resulted from the SPSRM (eq. 7). (*c*) The relationship between hake biomass and log(*R*/*S*). Dots (●) are the observed values, the straight line is the linear regression fit, and the curved line resulted from the SPSRM (eq. 7).

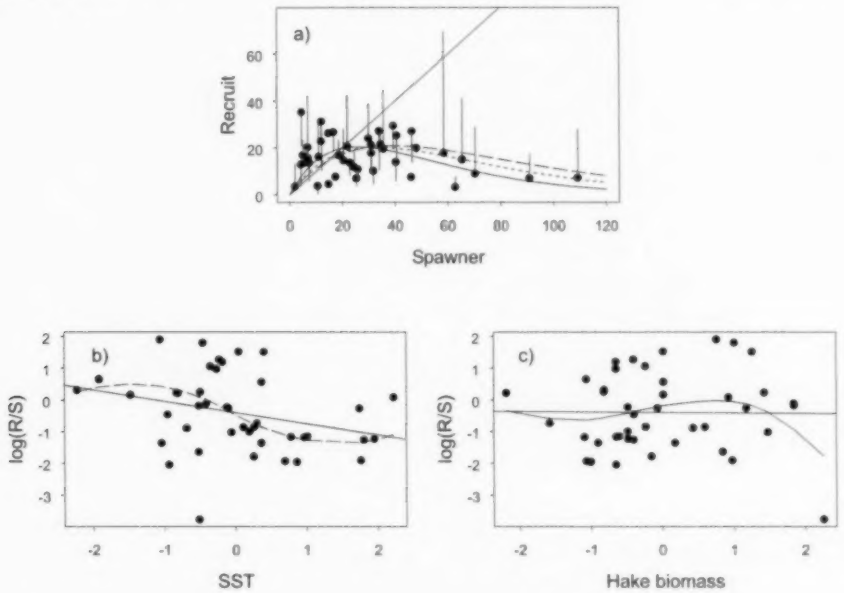
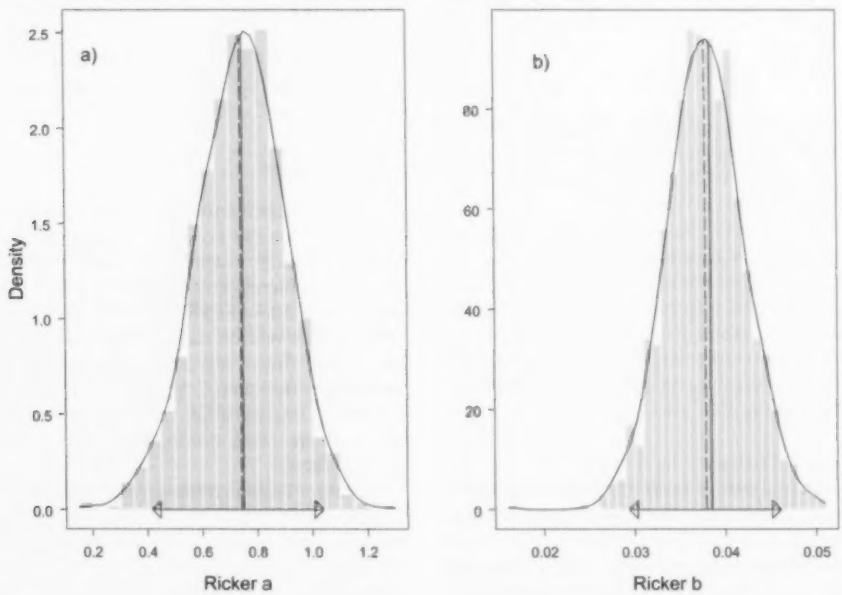


Fig. 5. Bootstrapping distributions for the stock–recruitment parameters *a* and *b* from West Coast Vancouver Island herring (1000 runs). For both plots, the curved line is the kernel density smoothing estimate. The sample mean and the observed value are marked as vertical broken and solid lines, respectively. The horizontal lines with open arrows on both ends indicate the 95% confidence intervals.



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with the survival of salmon, herring, and other species (Quinn and Deriso 1999; Ware 1991; Maunder 1998). Similarly, hake is a significant predator of herring (Chen and Ware 1999) and was included in our second example. Workers must resist the temptation to include environmental variables that appear to explain outliers in a stock–recruitment relationship but which may not be causal.

We found that a nonlinear empirical approach described the relationships between environmental variables (i.e., temperature and predator biomass) and recruitment better than a linear approach. While there was no obvious biological mechanism for these nonlinear relationships, using them allowed us to use our semiparametric approach. In each example, our SPSRM had a lower SSE and a higher R^2 value than either the traditional or the extended Ricker models, and hence we concluded that the SPSRM was superior. It is of course possible that there are parametric (and nonparametric) models that we have not tested that perform as well as the SPSRM.

Hilborn and Walters (1992) discussed the use of linear and nonlinear approaches to estimate the precision associated with stock–recruitment parameters and concluded that bootstrapping methods were among the most promising techniques. We used bootstrapping that allowed us to develop distribution plots for the stock–recruitment parameters productivity and capacity (i.e., a and b).

A manager can make a more informed decision when presented with a distribution plot than with, for example, a single-point parameter estimate. In the case of species that die after spawning, such as Pacific salmon, this is especially germane in that distribution plots can be produced for estimates of optimum stock sizes and harvest rates. A fishery manager concerned about overexploiting a stock or missing a potential fishing opportunity has a reduced probability of making an inappropriate decision when provided with information in a format such as this.

We emphasize that the environmental effects and fishery interventions are adjustment factors for traditional S–R analysis. Our semiparametric S–R model can be further generalized mathematically to include a nonparametric form for the spawner variable:

$$(15) \quad \log\left(\frac{R}{S}\right) = a + g(S) + f(Z)$$

where $g(S)$ is an arbitrary linear or nonlinear function. Furthermore, eq. 15 can be extended to the following form:

$$(16) \quad \mathrm{TR} = a + g(S) + f(Z)$$

TR can be any transformation of recruitment R. In their analysis of stock–recruitment for Pacific sardine (*Sardinops sagax*) off the west coast of North America, Jacobson and MacCall (1995) considered some of the special cases for eq. 16 with $\mathrm{TR} = \ln(R)$ and $F(Z) = \beta Z$ for the SST. These models were used to link sardine recruitment with their spawning biomass and SST. Accordingly, we believe that the semiparametric stock–recruitment model can be a useful tool for stock–recruitment relationship analysis for fish stocks.

In conclusion, the semiparametric model we developed, when used with bootstrapping to identify error structures, has advantages over, and may be more analytically sound than, parametric methods normally used. The SPSRM can uncover potential nonlinear relationships in stock recruitment analysis without the need to specify the functional form of the nonlinear relationships. Our approach can provide accurate parameter estimates and generate distribution plots for stock–recruitment and fishery management parameters that should reduce the risks associated with the management of valuable fishery resources.

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Annual variation in size-selective mortality of Atlantic salmon (*Salmo salar*) fry[1]

S.P. Good, J.J. Dodson, M.G. Meekan, and D.A.J. Ryan

Abstract: We investigated the size-selective mortality of Atlantic salmon (*Salmo salar*) fry during two consecutive summers that differed markedly in weather conditions. We sampled fry shortly after emergence in June and at the end of August to compare the distributions of back-calculated body size at hatching by examining otolith microstructure. Size-selective mortality was observed in both summers; however, the direction and strength of mortality differed. During the drought conditions of 1995, selective mortality was relatively weak and directed towards the smaller fry in the population. During the flood conditions of 1996, selective mortality was relatively strong and directed towards the larger fry of the same population. Interannual variability in size-selective mortality contributed to significant differences in the mean size of fry at the end of their first summer of life. Size-selective mortality rates estimated from the shifts in fish length at hatching observed during the first summer of life were comparable with published estimates of total mortality of Atlantic salmon fry, indicating that early mortality may be largely size selective. Mortality associated with hydroclimatic events can select against either small or large fish and is a key determinant of mean size attained by the end of the first summer of life.

Résumé : Nous avons étudié la mortalité sélective, en fonction de la taille corporelle, des alevins du saumon de l'Atlantique (*Salmo salar*) durant deux étés consécutifs dont les conditions météorologiques ont été très différentes. Nous avons échantillonnés des alevins après leur émergence au mois de juin et à la fin du mois d'août afin de comparer la distribution de leur taille corporelle à l'éclosion, retrocalculée après examen de la microstructure de leurs otolithes. L'intensité et la direction de la mortalité sélective selon la taille ont été différentes pour les deux années. Pendant les conditions de sécheresse en 1995, la mortalité sélective a été relativement faible et a affecté surtout les plus petits alevins de la population. Pendant les conditions de crue en 1996, la mortalité sélective a été plus forte et dirigée contre les alevins de plus grand taille de la même population. La variabilité inter-annuelle de la mortalité sélective a contribué aux différences significatives de la taille moyenne des alevins observée à la fin de leur premier été de vie. Les taux de mortalité sélective selon la taille, estimés à partir des changements de taille corporelle à l'éclosion entre juin et août, sont comparables aux estimations de la mortalité totale des alevins du saumon de l'Atlantique déjà publiées. Ceci suggère que la mortalité subie tôt dans la vie pourrait être sélective surtout en fonction de la taille. La mortalité associée aux événements hydroclimatiques peut être dirigée contre les petits ou les grands alevins et détermine la taille moyenne que les jeunes saumons peuvent atteindre à la fin de leur premier été de vie.

Introduction

It is generally thought that larger and faster growing juvenile fish are more likely to survive, with mortality being directed towards smaller, slower growing fish. Smaller fish are more susceptible to starvation and predation. Due to their lower social status and smaller size, they are often unable to successfully compete for food or shelter from predators (Miller et al. 1988). Predators of young fish are thought to preferentially seek out and consume smaller, more vulnerable prey (Parker 1971; Juanes 1994). On the other hand, predators may preferentially choose larger juveniles, possibly due to the higher visibility and larger net energy gains associated with consuming larger prey (Pepin and Shears 1995; Gleason and Bengtson 1996*a*, 1996*b*).

In the first few weeks after hatching and emergence from the redd, young salmonids undergo size-selective mortality. Evidence for this process is derived from two sources: firstly, studies that have followed the fate of marked or known individuals (e.g., Elliott 1990), and secondly, growth

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histories that have been back-calculated from otoliths of fish where a single population or cohort has been repeatedly sampled through time (e.g., West and Larkin 1987). In all cases, the selection documented by these studies has been unidirectional, so that salmonids that were relatively small at hatching have undergone higher mortality than those that were large at hatching.

Behavioural studies of growth and size-selective mortality in salmonids have focused on the role of dominance status and competitive ability. Larger, faster growing fish occupy and defend the most profitable territories in the stream, while slower growing fish are forced into less suitable habitats and may eventually die (Elliott 1989*a*, 1989*b*). This process may not only influence mortality rates, but also the life history patterns of surviving fish. Individuals that are superior competitors and thus grow fastest may migrate to sea after only 1 year in freshwater, while slower growing fish delay migration, remaining in freshwater for at least a second year (Thorpe 1977, 1989; Wright et al. 1990).

It is unlikely, however, that behavioural traits are the sole factors influencing mortality, as the environment experienced by young fish may be an important determinant of the intensity and nature of mortality. Numerous studies have shown that meteorological and hydrological events can create environments that are unfavourable to early life history stages, so that only certain size-classes of fish survive. For example, Uphoff (1989) found that rainfall during the early postlarval stage of striped bass (*Morone saxatilis*) increased mortality among smaller individuals. Similarly, Logan (1985) found that increased flow and decreased water temperatures in the Hudson River negatively affected the growth of larval striped bass. Fish that had not yet reached a critical body size for survival under these conditions suffered high levels of mortality. In contrast, Angermeier and Karr (1984) and Pearsons et al. (1992) suggested that during flood events, size-selective mortality of stream fishes might be directed towards larger members of a cohort, as they may be unable to occupy habitats that provide physical barriers to high water velocity.

At present, few studies have examined the importance of environmental conditions in determining the extent and direction of size-selective mortality during the first year of life of Atlantic salmon (*Salmo salar*). Here, we investigate the size-selective mortality of salmon fry during two consecutive summers that differed markedly in weather conditions. In order to detect selective mortality, we back-calculated fish size at hatching from the otoliths of salmon cohorts sampled shortly after emergence at the end of June and again in late August during the summers of 1995 and 1996. Shifts in distribution of size at hatching between June and August samples were used as evidence that the cohort had undergone selective mortality (e.g., West and Larkin 1987; Meekan et al. 1998*a*, 1998*b*).

Total mortality is composed of size-selective mortality and random mortality. Random mortality is the process of removing fish from the population that is not related to size. Few studies have attempted to evaluate the relative importance of these two sources of mortality in salmon populations. We estimated the levels of size-selective mortality required to produce the shifts in the distribution of size at hatching documented in our study and compared them with independent estimates of total mortality among salmon fry. Estimates of size-selective mortality rate comparable with independent estimates of total mortality rate would indicate that mortality was largely size selective. Such a demonstration would also refute the notion that shifts in the distribution of size at hatching between samples taken on two separate occasions may occur as a result of selection based on traits other than size (Mosegaard 1990; Metcalfe et al. 1992; but see Meekan et al. (1998*a*, 1998*b*) for an alternative view).

Materials and methods

Study site and sample collection

Atlantic salmon fry were sampled in the Ste-Marguerite River system in eastern Québec, Canada, during the summers of 1995 and 1996 (Fig. 1). Fry were collected from spawning/nursery grounds shortly after emergence and at the end of the summer. Spawning ground substrate was composed primarily of small gravel particles (diameter ranging from 20 to 30 mm) and of dispersed small boulders located mainly at the tails of pools and near the beginning of riffles. They are characterized by shallow (10–40 cm deep) and moderately fast running (50 $cm \cdot s^{-1}$) water (reviewed in Stanley and Trial 1995). Nursery grounds are located in slower running water (end of riffles) where food is abundant and well distributed in the water column. Sampling was conducted at three spawning/nursery sites in 1995 and five such sites in 1996, two of which corresponded to sampling locations in 1995. In 1995, the three sites were located on the northeast branch at kilometre 6 (NE 06) just below a migratory fish ladder and on the principal branch at kilometre 35 (PR 35) and kilometre 58 (PR 58). In 1996, sites NE 06 and PR 58 were resampled. The spawning/nursery site located at PR 35 was abandoned in 1996, as stocking of fry produced in an in situ incubator may have confounded comparisons of fish length distributions at hatching calculated for fry sampled at emergence and again at the end of the summer. We thus sampled downstream at the next spawning/nursery area located at kilometre 27 (PR 27). New sites were added on the northeast branch at kilometre 28 (NE 28) just below the upstream limit of salmon migration and on the principal branch at kilometre 81 (PR 81). At each site, 20 fry were collected shortly after emergence at the end of June and then again at the end of August by electrofishing a 100-m^2 grid (5 × 20 m) laid out over the spawning/nursery ground to cover riffle and channel habitats. If 20 fry were not captured, a second 100-m^2 grid was laid out. In the laboratory, fish were measured using a dissecting microscope and image analysis system.

Otolith preparation

Left and right sagittal otoliths were removed from fish under a dissecting microscope equipped with a polarized light source. Otoliths were cleaned and glued to glass slides with Super Glue®. Once dry, they were ground on lapping film to the plane of the core and viewed using a compound microscope linked to an image analysis system (OPTIMAS). Growth increments were counted and measured along a constant axis from the centre to the edge of the otolith. The area within the hatch mark and the total area of the otolith were also measured.

Data analysis

Backcalculation and size-selective mortality

The standard lengths of young salmon at hatching and yolk sac absorption were back-calculated from fish collected in June and August of 1995 and 1996 using the biological intercept technique (Campana and Jones 1992). The assumptions of the back-calculation technique (daily increment formation and proportional-

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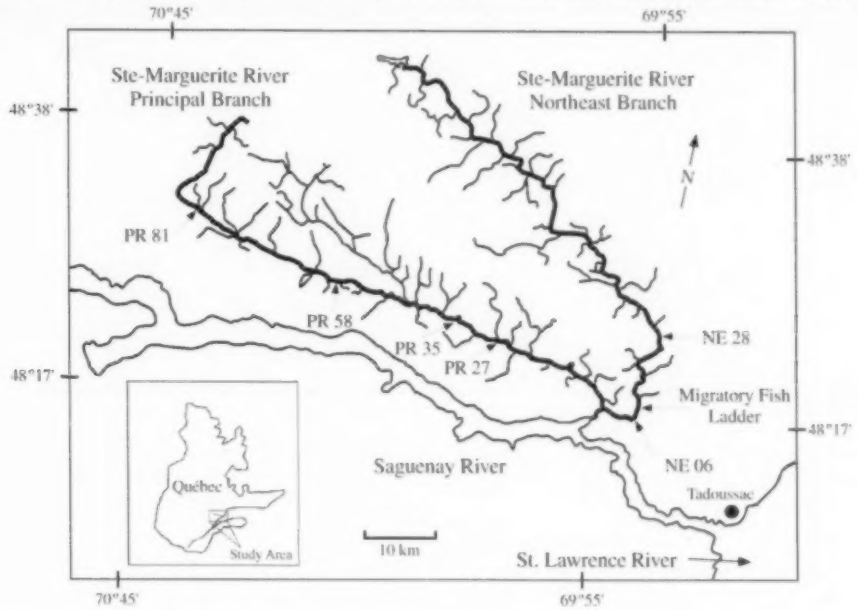
Fig. 1. Location of the Ste-Marguerite River, Québec, Canada, identifying the sites sampled in the summers of 1995 and 1996.



ity between the otolith and somatic growth) have previously been validated for Atlantic salmon fry in the weeks immediately following hatching by Meekan et al. (1998*a*). They also demonstrated that distinctive checks are laid down on the otoliths of young salmon at hatching and then again at yolk-sac absorption, allowing the timing of these events to be determined. In young Atlantic salmon approaching the end of their first summer of life, daily increment formation and proportionality between otolith and somatic growth were validated by Wright et al. (1990). However, they also found that otolith and somatic growth might not be proportional in fish undergoing smoltification. In the Ste-Marguerite River system, no young salmon undergo smoltification at the age of 1+.

A two-factor analysis of variance (ANOVA) was used to evaluate changes in size-selective mortality between years. Site was included as a blocking effect, allowing for consistent differences in size at hatching between sites. Year (1995 or 1996), sampling period (June or August), and the interaction between year and sample period were included to test for changes in the size at hatching due to the main effects of year, sampling period, and, most importantly, the interaction between year and sampling period. The interaction is the most important term because it tests for changes in selective mortality between years adjusted for differences between sites. Comparisons of the means were carried out using the pairwise *t* tests at the 5% level of significance, only if the overall comparison of the interaction or main effects were significant at the 5% level (Steel and Torrie 1981). A residual analysis was carried out to ensure that there was no evidence that the assumptions of ANOVA were violated.

Generation of survivorship curves and mortality estimates

Frequency distributions of size at hatching of fish collected in June and August were modelled as the product of two multinomial distributions:

$$L(\pi_{01},\ldots,\pi_{0k},\pi_{11},\ldots,\pi_{1k}) = \binom{n_0}{y_{01},\ldots,y_{0k}}\pi_{01}^{y_{01}}\ldots\pi_{0k}^{y_{0k}} \cdot \binom{n_1}{y_{11},\ldots,y_{1k}}\pi_{11}^{y_{11}}\ldots\pi_{1k}^{y_{1k}}$$

where π_{ij} is the probability of a fish occurring in the *j*th size-class at hatching ($j = 1,\ldots, k$), k is the number of size-classes in the *i*th time period ($i = 0$, prior to the period of interest, or $i = 1$, after the period of interest), n_i is the total number of fish captured in the *i*th period, and y_{ij} is the number of fish captured in the *i*th time period that were in size-class *j* at hatching. Since the same cohort was sampled in both time periods, the probability of a fish originating from hatch size-class *j* at time 1 must be related to the probability of a fish originating from hatch size-class *j* at time 0 and the probability of a fish surviving. The relationships between these variables were modelled as

$$\pi_{1j} = \frac{\pi_{0j}S_jR}{\sum_{j=1}^{k}\pi_{0j}S_jR}$$

where S_j represents the probability that a fish of hatch size *j* survives all size-selective mortality and R represents the probability that a fish survives all nonselective (random relative to the size of fish at hatching) mortality that occurs during the period of observation. For the purposes of this study, S_j was modelled as a monotonic sigmoid curve:

$$S_j = \frac{e^{\alpha+\beta x_j}}{1+e^{\alpha+\beta x_j}}$$

where the parameters α and β corresponded to the level and rate of change of the curve, respectively. In this case, a large positive value of β indicates a severe positive size-selective process, while a zero value of β indicates that mortality is not size selective.

In this model, only the product of S_jR can be estimated, since without knowledge of the process causing the death of an individual fish, S_j and R cannot be estimated. However, if it is assumed that $R = 1.0$ (i.e., that the cohort undergoes only size-selective mortality), then the maximum amount of selective mortality that can occur for a given size-class (S_j'') can be estimated.

Survivorship curves and estimates of size-selective mortality were modelled for the data sets from each site in both years of the study as well as for data sets pooled within years. From each of

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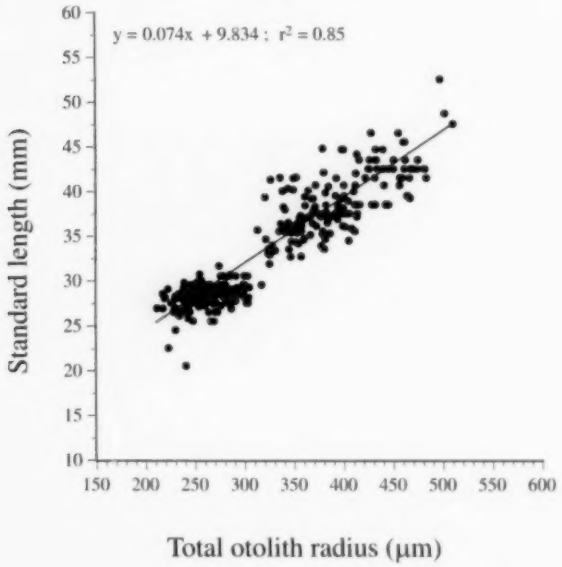
Table 1. Average monthly precipitation, river discharge, and air temperature for the Ste-Marguerite River.

	Average precipitation (mm)			Average discharge ($m^3 \cdot s^{-1}$)			Average temperature (°C)		
	1974–1994	1995	1996	1974–1994	1995	1996	1974–1994	1995	1996
Jan.	78.9	107.0	74.0	7.56	11.81	85.31	–10.9	–8.4	–12.4
Feb.	62.5	89.0	126.0	6.11	8.43	75.40	–8.4	–10.2	–9.7
Mar.	56.7	99.0	10.0	5.94	5.90	67.24	–5.4	–5.7	–5.0
Apr.	71.4	102.0	142.4	44.71	23.96	102.16	7.6	5.7	7.4
May	103.2	115.2	89.8	111.59	134.72	135.26	15.4	15.2	16.1
June	105.5	43.0	91.6	42.13	41.01	na	20.8	24.9	23.4
July	131.8	83.0	303.7	29.02	12.17	148.82	23.2	26.2	22.9
Aug.	112.5	160.0	29.6	22.44	6.04	29.08	21.8	25.1	24.7
Sept.	117.7	113.3	149.2	22.17	8.85	36.03	15.6	16.1	18.2
Oct.	105.9	99.7	135.0	32.95	33.54	na	8.8	12.3	9.0
Nov.	93.8	62.0	131.4	25.12	50.17	na	4.0	4.2	5.8
Dec.	99.2	43.0	114.0	14.12	87.11	na	–7.5	–7.4	–4.9

Note: May–August represent summer values. na, not available.

Fig. 2. Correlation between standard length of Atlantic salmon fry and otolith radius for 1995 and 1996.



these data sets, 1000 bootstrap samples were generated and the size-selective survivorship curve estimated using a grid search (Draper and Smith 1981) for the parameters α and β. The parameters π_{ij} were not included in this process, as this would have required a grid search of 14 parameters, which was beyond the computational resources available to the study. While alternative techniques of maximizing likelihoods were examined, (e.g., gradient methods), these were rejected because they failed to converge on estimates.

The maximum mortality (MM) undergone by a cohort was estimated from the following equation:

$$MM_j = \frac{\sum_{j=1}^{k} S_{ij}^{\alpha} \pi_{0j}}{\sum_{j=1}^{k} \pi_{0j}}.$$

Estimates were calculated for each of the 1000 bootstrap samples and the median, 5th, and 95th percentiles then calculated for each site and for each year averaged among sites.

Results

Environmental conditions

Weather patterns during the summers of 1995 and 1996 were among the most distinct ever recorded in the Saguenay – Lac St. Jean region of Québec (Table 1). Drought-like conditions occurred during much of the spring and summer of 1995. Air temperatures in June through August were several degrees higher than the average for the previous 20 years (1974–1994). Average monthly precipitation was low, especially during June and July, resulting in a severe reduction in river flow from mid-June until the end of September. In contrast, the largest flood event in Québec's recorded history occurred on July 20, 1996. In 1996, rainfall for the month of June was more than double that of June 1995, and July's rainfall was almost four times that of July 1995 and twice that of the previous 20-year average. As a result, river discharges for July and August 1996 were 12 times and five times more than in July and August 1995, respectively (Table 1).

Otolith and somatic growth

There was a strong correlation between otolith radius and standard length of young salmon in both years of sampling. As an analysis of covariance found no significant differences between regression lines calculated for each year ($p = 0.35$), data sets were pooled and a single regression calculated ($r^2 = 0.85$, $n = 316$) (Fig. 2).

Variability in size-selective mortality

Standard lengths at hatching were back-calculated for fish collected in June and August for both 1995 and 1996. Back-calculation results revealed significant shifts in standard length and otolith radius at hatching between the June and August samples for both years of sampling (Fig. 3). In 1995, mean otolith radius at hatching of fish sampled in June was 93.26 μm (SE = 1.7 μm), while August fish had a larger mean otolith radius at hatching of 99.64 μm (SE = 1.7 μm). During the following summer, this pattern was reversed, with the otolith radius at hatching of fish collected in June averaging

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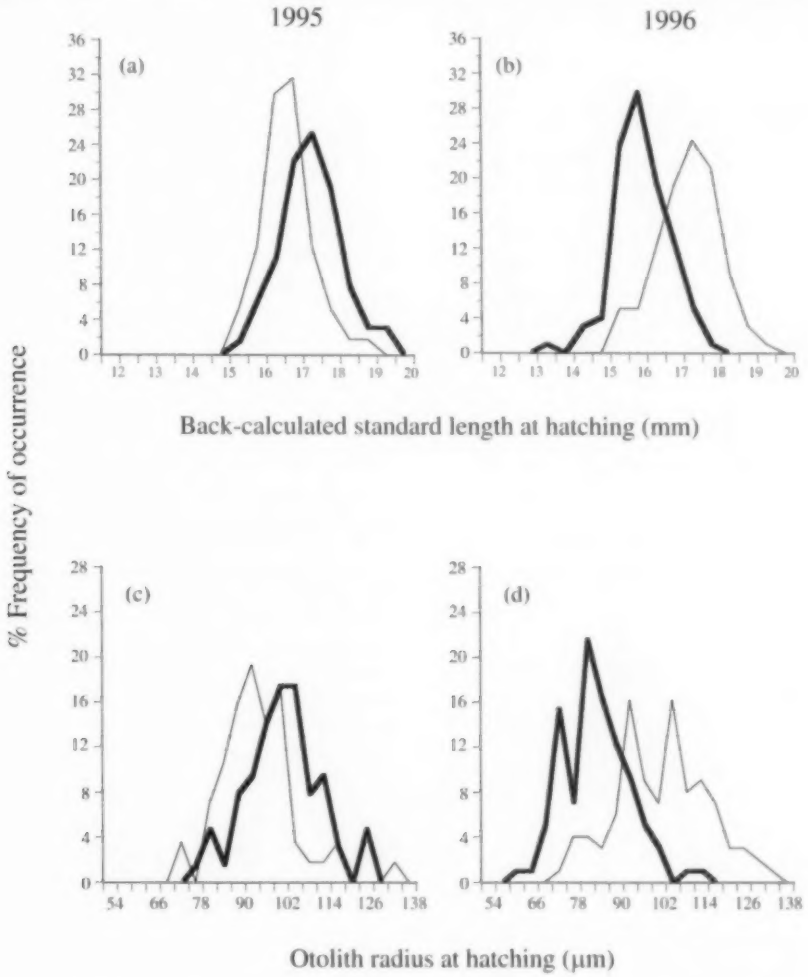
Fig. 3. Comparisons between the size distributions of (*a* and *b*) standard length and (*c* and *d*) otolith radii at hatching for Atlantic salmon fry sampled in June (thin lines; $n = 57$ in 1995 and $n = 99$ in 1996) and August (thick lines; $n = 63$ in 1995 and $n = 97$ in 1996).



100.9 μm (SE = 1.2 μm) and of those collected in August averaging 82.3 μm (SE = 1.2 μm) at hatching. Corresponding shifts were also recorded in average standard length at hatching between these months (Fig. 3). The two-factor ANOVA revealed significant changes in back-calculated otolith radius and fish length due to the interaction between year and sampling period ($p < 0.0001$). There were thus significant changes in selective mortality between years adjusted for differences between sites. Mean size (otolith radius and standard length) at hatching for fish collected in June and August were significantly different in both years ($p < 0.001$).

The change in the direction of size-selective mortality of salmon fry from 1995 to 1996 contributed to large differences in the size distributions of fry at the end of the summer (Fig. 4). The 1995 cohort attained a significantly greater mean size by the end of their first growing season than did the 1996 cohort (ANOVA, $p < 0.0001$).

Survivorship curves and mortality rates

Patterns in survivorship were consistent among sites within each year (Table 2; Fig. 5). The slope of survivorship curves tended to be more gentle in 1995 than in 1996, indicating that the gradient in survivorship between large and small individuals (i.e., size selectivity) was relatively weak in 1995. When size-selective mortality is weak, a small proportion of the bootstrapped estimates of size-selective mortality rates may be close to zero. As a result, the distribution of mortality estimates in 1995 was strongly bimodal for PR 35 and weakly so for NE 06, the first mode being close to zero. As a result, large error estimates for survivorship curves and mortality rates were estimated in 1995 (Table 2; Fig. 5). Therefore, although mortality rates estimated from combining all sites sampled in 1995 revealed that fry suffered a median mortality rate of 58% between June and August (Table 2), the 5th and 95th percentile limits indicated that size-selective mortality rates between 26 and 78% could have been responsible for the observed shifts in otolith size at hatching between June and August.

In contrast, the slope of survivorship curves tended to be more pronounced in 1996 than in 1995, indicating that size-

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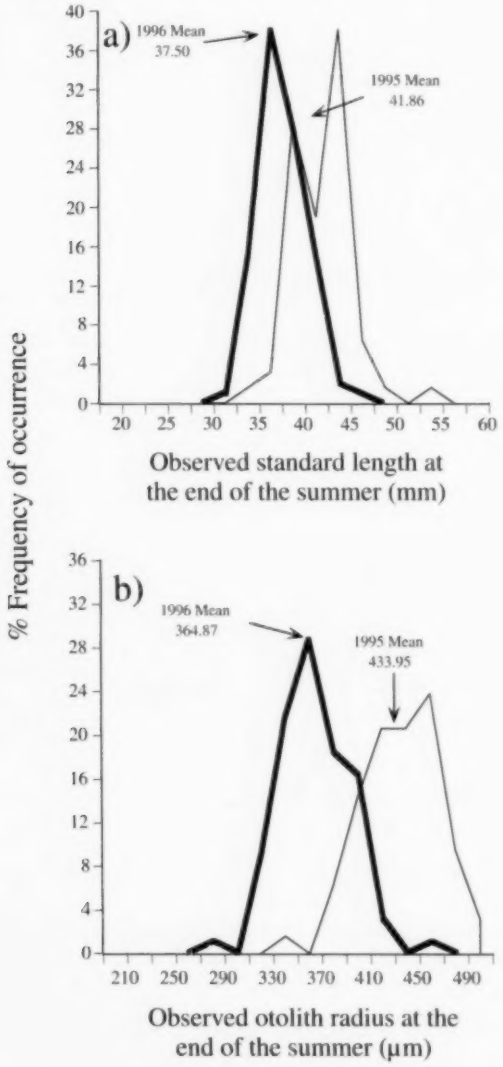
Fig. 4. Percent frequency of occurrence for Atlantic salmon (*a*) standard lengths and (*b*) otolith radii observed at the end of the summer (August) for both 1995 and 1996 sampling periods. The thin lines represent 1995 (n = 63) and the thick lines represent 1996 (n = 97).



selective mortality was relatively stronger in 1996. The distributions of bootstrapped estimates of size-selective mortality rates for individual and combined sites were unimodal and error estimates were narrower for survivorship curves and mortality rates calculated from sites sampled in 1996 (Table 2; Fig. 5). Overall estimated mortality rate in 1996 revealed that the cohort suffered a median size-selective mortality rate of 82% between June and August (Table 2). The 5th and 95th percentile limits indicated that size-selective mortality rates between 72 and 88% could have been responsible for the observed shifts in otolith size at hatching between June and August 1996.

Table 2. Estimated summer mortality rates (median, 5th, and 95th percentiles) of Atlantic salmon fry sampled at three sites in 1995 and five sites in 1996 and overall estimates within years in the Ste-Marguerite River.

Site	Year	Median	5%	95%
NE 06	1995	0.592	0.050	0.857
PR 58	1995	0.594	0.260	0.864
PR 35	1995	0.740	0.000	0.894
Overall	1995	0.581	0.266	0.789
NE 06	1996	0.732	0.470	0.906
PR 58	1996	0.814	0.608	0.948
PR 27	1996	0.979	0.951	0.994
PR 81	1996	0.818	0.629	0.954
NE 28	1996	0.802	0.395	0.949
Overall	1996	0.818	0.729	0.884

Discussion

High levels of size-selective mortality occurred among the fry of Atlantic salmon sampled in the Ste-Marguerite River during the summers of 1995 and 1996. However, the direction of size-selective mortality reversed over the two years of the study. In 1995, salmon fry sampled in August were significantly larger at hatching than those sampled in June, indicating that mortality rates were greater for the smaller individuals in the population. This observation conforms to the widely held view that large size is more advantageous to survival of young fish than small size (e.g., the bigger-is-better hypothesis; Miller et al. 1988). It is also consistent with the observation that mortality rates were greater for the smaller individuals of a cohort of sockeye salmon (*Oncorhynchus nerka*) (West and Larkin 1987; Meekan et al. 1998*b*). However, the bigger-is-better hypothesis was not supported by results from the summer of 1996. In that year, fish collected in August were significantly smaller at hatching than those collected in June, implying that there was relatively strong selection against larger individuals over the summer of 1996.

Gleason and Bengston (1996*a*) provided additional evidence that bigger is not necessarily better for the survival of young fishes. These workers found that size-selective predation by juvenile bluefish (*Pomatomus saltatrix*) was consistently directed towards the larger individuals of a population of inland silverside (*Menidia beryllina*). Their results imply that the outcomes of size-selective mortality may vary due to the identity of the prey and the foraging tactics of the predator. The results of our study are novel, however, in that they demonstrate that the direction of size-selective mortality can also differ between years for the same population.

As patterns of survivorship were consistent among sites within years, the contrasting patterns of size-selective mortality documented between 1995 and 1996 occurred at the scale of the entire river. For this reason, it seems likely that the major changes in weather and river flows that occurred between the two years were responsible for the different mortality patterns. Numerous studies have demonstrated that river flow is an important factor directly influencing the survival of the early life history stages of fluvial fishes. Crecco

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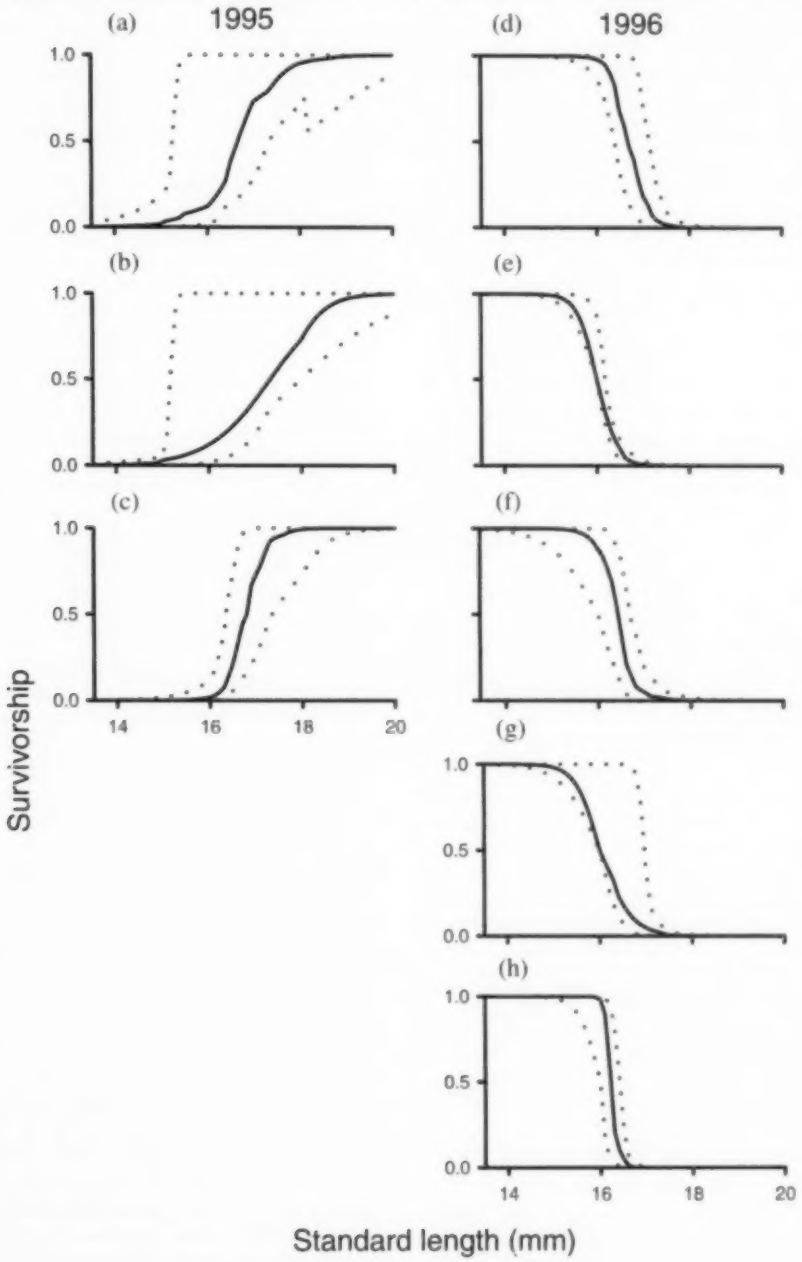
Fig. 5. Estimated survivorship curves of Atlantic salmon fry sampled from the Ste. Marguerite River in 1995 and 1996. (*a*) NE 06; (*b*) PR 35; (*c*) PR 58); (*d*) NE 06; (*e*) PR 27; (*f*) PR 58; (*g*) NE 28; (*h*) PR 81. Dotted lines represent the 5th and 95th percentiles.



and Savoy (1987) showed that temporal changes in river flow and temperature coincident with early larval development regulated the feeding success of American shad (*Alosa sapidissima*). They found that 80–87% of the annual variability in recruitment over a 14-year period was explained by variability in May and June River flows and monthly rainfall. In the case of Atlantic salmon, river discharge during the weeks and months following emergence influences fry survival and year-class strength (Frenette et al. 1984; Gibson and Myers 1988; Jensen and Johnsen 1999).

It is possible that the unusually dry conditions of 1995 may have accentuated mortality among the smallest salmon fry. Tremblay et al. (1993) studied habitat use by Atlantic salmon fry in the Trinité River on Québec's North Shore. They found that habitat preferences were strongly size dependent. Small salmon fry (30–40 mm) occupied shallow

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riffle areas, while larger fry (40–50 mm) occupied the deeper channels. In the Ste-Marguerite River, low-flow conditions in 1995 caused riffles to become shallower than normal, or even completely exposed. The smaller fry that occupied these areas may have been forced into deeper channels with the larger fry. More intense competition between salmon in these habitats may have increased the mortality rate of smaller, competitively inferior fish.

In contrast, smaller fry had the advantage during the major flood of 1996. During floods, fish may seek refuges that provide barriers to high water velocity (Angermeier and Karr 1984; Pearsons et al. 1992; Lonzarich and Quinn 1995). We know from trap net samples taken in the estuary that large numbers of 1+ and 2+ parr were swept out of the Ste-Marguerite River by the 1996 flood (J. Dodson, CIRSA, unpublished data). Large fry may have suffered a similar fate but were too small to be captured in our trap nets. As small fry may have already been associated with the shallowest riffles, these fish may have been spared the strongest currents occurring in deeper channels. Alternatively, they may have been able to exploit refuges from the current that were unavailable to larger fish due to their size.

Irrespective of the mechanism involved, it is evident that selective mortality can influence the mean length attained by young salmon at the end of the summer and thus their recruitment to later life history stages. There is evidence that this process plays a similar role in other species. Cargnelli and Gross (1996) found that overwinter survival of large-bodied young-of-the-year bluegill (*Lepomis macrochirus*) was 231% higher than for fish that were comparatively small at the end of their first summer of life. This survival translated into higher recruitment rates of large individuals to the population the following year. Similarly, Griffiths and Kirkwood (1995) found that overwinter survival of roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) was highly dependent on size at age at the end of the summer.

The apparent difference in growth rate that we observed between years was a reflection of the size-selective mortality that was imposed each year. It appeared that fish sampled in 1995 had higher growth rates than fish in 1996; however, selection was against smaller fish in 1995 and larger fish in 1996. Therefore, growth rates were high in 1995 because larger, faster growing fish survived, while in 1996, growth rate was low because the small fish were the survivors. Although variables such as temperature and food availability influence growth, individual members of a cohort sampled at the end of the growing season are the survivors of size-selective mortality imposed on the population at an earlier time.

Heritability for growth rate in salmonid fishes ranges from 0.2 to 0.3, with a coefficient of variation of 20–30%, implying that it is possible to obtain large responses from selection for growth (Gjedrem 2000). The maintenance of high variance in growth rates of wild Atlantic salmon despite the potential for large responses from selection for growth may in part be related to the observation that survival advantages accrue to either faster or slower growing fish depending on environmental conditions.

The levels of size-selective mortality estimated from the shifts in otolith size and fish length at hatching observed during the first summer of life in 1995 and 1996 are comparable with independent estimates of total mortality rate. Direct estimates of total mortality of salmon fry are available for fry that have been stocked as part of restoration and enhancement programs for Atlantic salmon. In a tributary stream of the Connecticut River, fry measuring between 25 and 32 mm released in May at densities of between 100 and 150 fry·100 m^{-2} experienced summer mortalities of 65% in 1982, 98% in 1983, 82% in 1984, and 72% in 1985 (Orciari et al. 1994). Excluding the very high mortality of 1983 associated with the stocking of fry from an Icelandic stock, the mean summer mortality of stocked Penobscot-strain fry measured over a period of 3 years was 73% (SD = 8.5%). In the West River, Vermont, fry measuring 25–30 mm were released during May in successive years at densities ranging from approximately 50 to 150 fry·100 m^{-2}. These cohorts experienced summer mortalities of 85% in 1984, 94% in 1985, and 83% in 1986 (McMenemy 1995). Stocking at much lower densities (approximately 30 fry·100 m^{-2}) resulted in a mean summer mortality of 58% (SD = 8.1%) over a 5-year period. It is obvious that comparisons between these measurements and our estimated mortality rates are confounded by various factors (e.g., differences in years of observation and environmental conditions, the use of hatchery-reared as opposed to naturally produced fry, and different dates of sampling and release). Nevertheless, in the absence of more appropriate in situ measurements of fry mortality, size-selective mortality rates estimated from the shifts in otolith and fish size distributions at hatching documented in the present study were within the range of total mortalities measured in the field. The approximate agreement between observed total mortalities and our estimates of maximum size-selective mortality indicates that total mortality rates of salmon fry may be largely size selective.

Our results show that the prevailing view that size at age in young salmon is largely controlled by competitive ability acting on growth rate is overly simplistic. Regardless of inherent superiority, mortality associated with hydroclimatic events may select against either small or large fish and is a key determinant of mean size attained by the end of the first summer of life and subsequent recruitment to the older life history stages of Atlantic salmon.

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Productivity and survival rate trends in the freshwater spawning and rearing stage of Snake River chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract: Stream-type chinook salmon (*Oncorhynchus tshawytscha*) populations in the Snake River (northwest United States) have declined dramatically since completion of the federal hydrosystem. Identifying the life stage that is limiting the survival of these stocks is crucial for evaluating the potential of management actions to recover these stocks. We tested the hypothesis that a decrease in productivity and survival rate in the freshwater spawning and rearing (FSR) life stage since completion of the hydropower system could explain the decline observed over the life cycle. The decline of chinook populations following completion of the hydrosystem was not accompanied by major survival changes in the FSR life stage. FSR productivity showed no significant decline, and the FSR survival rate decline was small relative to the overall decline. However, significant survival declines did occur in the smolt-to-adult stage coincident primarily with hydrosystem completion, combined with poorer climate conditions and possibly hatchery effects. Potential improvements in survival that occur only at the FSR life stage are unlikely to offset these impacts and increase survival to a level that ensures the recovery of Snake River stream-type chinook.

Résumé : Les populations du Saumon quinnat (*Oncorhynchus tshawytscha*) de type riverain de la rivière Snake du nord-ouest des États-Unis ont décliné de façon spectaculaire depuis l'achèvement de l'hydrosystème fédéral. L'identification du stade du cycle biologique qui limite la survie de ces stocks est une étape cruciale dans l'évaluation du potentiel des aménagements nécessaires pour la récupération de ces populations. Nous posons en hypothèse que la diminution de la productivité et du taux de survie dans la partie du cycle relié à la fraye et au développement en eau douce (stade FSR), depuis l'achèvement du système hydroélectrique, peuvent expliquer le déclin observé dans tout le cycle biologique. Le déclin des populations de Saumons quinnat après le parachèvement de l'hydrosystème n'a pas été marqué par des changements majeurs de la survie durant le stade FSR; la productivité durant ce stade n'a pas baissé de façon significative et la réduction des taux de survie a été faible par comparaison au déclin total. Cependant, des réductions significatives de la survie se sont manifestées durant le passage du stage saumonneau au stade adulte qui ont coïncidé particulièrement avec l'achèvement de l'hydrosystème, mais aussi avec des conditions climatiques défavorables et peut-être aussi avec des effets de pisciculture. Des améliorations potentielles de la survie au seul stade FSR sont donc peu susceptibles de compenser les impacts et d'augmenter la survie à un niveau qui assure la récupération des stocks de Saumons quinnat de type riverain de la rivière Snake.

[Traduit par la Rédaction]

Introduction

The Columbia River Basin once contained extremely abundant runs of chinook salmon (*Oncorhynchus tshawytscha*) that supported a substantial freshwater harvest (Chapman 1986). Over the last century, however, these stocks, in addition to sockeye (*Oncorhynchus nerka*) and steelhead (*Oncorhynchus mykiss*), have declined dramatically in response to a combination of factors including extensive hydroelectric development, harvest, habitat degradation, and emphasis on mitigating anthropogenic losses with hatchery releases. Snake River spring and summer chinook populations, once the predominant run of stream-type chinook in the Columbia River Basin, have declined dramatically since completion of the Federal Columbia River Power System (hereafter referred to as the hydrosystem). They have been listed as threatened under the Endangered Species Act since 1992. To address this population decline, the National Marine Fisheries Service's 1995–1998 biological opinion on operation of the hydrosystem created a process called PATH (Plan for Analyzing and Testing Hypotheses) (Marmorek et al. 1998). The PATH process evaluated hypotheses about the distribution of mortality over the life cycle and strengths and weaknesses of supporting evidence and tested those alternative hypotheses that had significant management implications (Marmorek et al. 1998).

The PATH modeling forum was a critical component of the evaluation of the effects of breaching four large hydroelectric dams on the lower Snake River for salmon recovery,

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and several important conclusions regarding the survival of chinook evolved from the PATH retrospective analyses that will help decision makers better evaluate options for recovery. Schaller et al. (1999) demonstrated that productivity and survival rates of Snake River stream-type chinook declined and also became more variable following the completion of the hydrosystem. Declines in survival rates for Snake River stocks were also greater than for similar stocks, which migrate past fewer dams (Schaller et al. 1999; Deriso 2001). Both of these analyses were based on spawner (parent) and recruit (progeny) data, which provide information about the overall survival of a salmon stock. However, in addition to considering the overall patterns, it is important to consider survival through specific life stages in order to identify which life stages are limiting the survival and recovery of a threatened population. For the Snake River, the National Marine Fisheries Service recently suggested that hydrosystem impacts might be mitigated through habitat improvements in combination with other nonhydro changes (i.e., offsite mitigation) (National Marine Fisheries Service 2000). These potential habitat improvements would hypothetically increase productivity and survival rates during the freshwater life stage occurring between spawning and the beginning of smolt migration to the ocean. However, if the proposed management actions do not address limiting factors, the populations will continue to face high extinction risk and will have a low chance of recovery.

If the pattern of decline for Snake River spring and summer chinook is largely due to the degradation of habitat conditions, we would expect to see a decrease in productivity and survival rate at the freshwater life stage. The parent–progeny approach used here (i.e., analysis of spawner and smolt patterns) evaluates whether productivity and survival rates changed in the freshwater stage for the aggregate Snake River spring and summer chinook population since the 1960s. In addition, we then evaluated whether the effects of habitat degradation or other influences early in the life cycle (e.g., hatchery effects) since the 1960s could explain a change in productivity and survival at the freshwater life stage. A broad mix of land use influence, from minor (wilderness) to management for irrigated agriculture, livestock grazing, logging, and mining, existed throughout the time series (Fulton 1968; Beamesderfer et al. 1997). However, a majority of the land use impacts in many Snake River drainages occurred prior to the time period of lower Snake River dam construction and completion (Thompson and Haas 1960; Fulton 1968). A decrease in freshwater spawning and rearing (FSR) productivity and survival rate after completion of the hydrosystem might be considered evidence for an overall decrease in habitat quality, or simply the occurrence of unfavorable environmental conditions (e.g., drought) during this life stage, negative effects of hatchery fish (e.g., competition), or depensatory survival mechanisms (Dennis 1989) expected at the low observed spawner levels.

In this paper, we present a temporal comparison of FSR productivity and survival rate patterns from the aggregate run of naturally spawning Snake River spring and summer chinook, brood years 1962–1997. Spawner (parent) and smolt (progeny) estimates are used to evaluate whether there has been a net decrease in FSR productivity and survival rate during the FSR stage for Snake River spring and summer chinook. We also evaluate whether a decrease, if detected, could be associated with a temporal change in habitat conditions across the landscape of stocks. Finally, we attempt to determine if a change in freshwater productivity and survival rates could explain the corresponding decline in adult productivity and survival rates (over the life cycle) observed since completion of the hydrosystem.

Methods

Estimates of FSR survival were based on counts of returning spawners and smolts at the uppermost dam (Fig. 1). Counts and estimates of freshwater survival were for the aggregate of naturally spawning Snake River spring and summer chinook including stocks from streams in Idaho and Oregon, upriver from Lower Granite Dam. Spawning takes place primarily in August through September. The eggs are deposited and fertilized by spawning adults, the embryos incubate within the gravel substrate and then hatch, and the fry emerge into the freshwater habitat. The juvenile fish rear in headwater streams (stream-type chinook), producing yearling smolts, which migrate seaward in the spring (primarily April and May) nearly 2 years after egg deposition.

Returning adult spawners migrate through the eight hydroelectric dams on the lower Columbia River and Snake River and are counted at each dam during passage at ladders. Counts at the uppermost dam (Lower Granite Dam since 1975) provide estimates of the number of adults returning to spawn in the Snake River each year. Similarly, smolts are counted at Lower Granite Dam during their downstream migration. The number of smolts from each parent brood of spawners that survives to Lower Granite Dam provides a measure of survival and productivity in upstream spawning and rearing areas (smolts per spawner) (Fig. 1). The aggregate population of spring and summer chinook counted at Lower Granite Dam includes stocks that spawn in streams from a broad mix of habitat conditions ranging from wilderness areas (Minam River, Oregon, and Sulphur Creek, Idaho) to heavily impacted areas (Poverty Flat and Bear Valley Creek, Idaho) (Fulton 1968; Quigley et al. 1999; Schaller et al. 1999).

Freshwater spawning and rearing life stage

We define FSR productivity, for a specified time period, as the natural log of the ratio of smolts per spawner at very low abundance of spawners (i.e., in the absence of density-dependent mortality) (Schaller et al. 1999). In addition, survival rate indices for the FSR stage provide a time series of density-independent survival estimates through deviations of observed ln(smolts/spawner) at a spawner level from that predicted by the fitted parent–progeny (Ricker spawner–recruitment) function for a specified time period (Schaller et al. 1999).

Wild spawner (parent) estimates

In this evaluation, spawners were defined as adult spring and summer chinook counted at the uppermost Snake River dam. Spawners included wild- and hatchery-origin fish spawning in tributaries. The uppermost dam changed during this era as new projects were added: Ice Harbor in 1961, Lower Monumental in 1969, Little Goose in 1970, and Lower Granite in 1975 (Fig. 1).

Dam counts used to derive spawner (S) estimates included data for brood years 1962–1997. The dam counts are total numbers of adult (ages 4–5) spring and summer chinook for wild- and hatchery-origin adults combined. The wild component of S (S_w) was estimated by subtracting tributary harvest of wild fish and the total hatchery run size from the combined wild and hatchery dam count:

$$(1) \qquad S_w = U_{count} - W_{har} - \frac{R_{rtn} + H_{har}}{S_{ps}}$$

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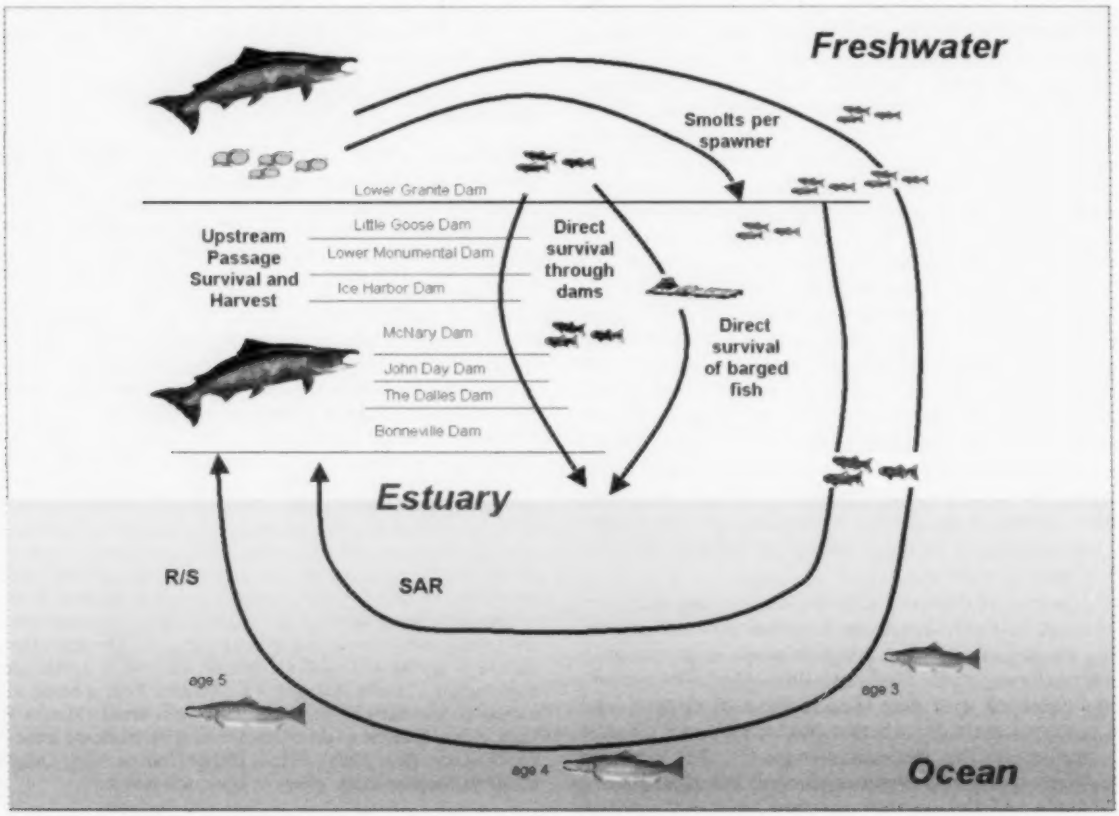
Fig. 1. Schematic life cycle diagram for Snake River salmon and steelhead showing passage past the dams and the different indices of survival across different life stages. Smolts per spawner provides an estimate of FSR survival, SAR is the survival rate of smolts to returning adults (smolt-to-adult), measured at the Columbia River mouth, and *R/S* is recruits per spawner, measured by back-calculating recruits (progeny) from spawners (parents) based on redd counts, age structure, and harvest.



where U_{count} is the total (wild and hatchery) adult spring and summer chinook count at the uppermost dam in the Snake River, R_{rtn} is the adult hatchery fish return to hatcheries for spring and summer chinook above the uppermost dam in the Snake River, W_{har} is harvest of adult wild spring and summer chinook above the uppermost dam in the Snake River, H_{har} is harvest of adult hatchery spring and summer chinook above the uppermost dam in the Snake River, and S_{ps} is prespawning survival rate (=0.8).

Columbia River management agencies provided estimates of W_{har}, R_{rtn}, and H_{har} each year since 1979 and assumed that S_{ps} was 0.8 for hatchery fish (Washington Department of Fish and Wildlife and Oregon Department of Fish and Wildlife 1999). We estimated S_w for the earlier brood years, 1962–1978, using similar methods. Snake River sport harvest estimates for the earlier brood years were based on Beamesderfer et al. (1997).

The number of wild smolts estimated at the uppermost dam included progeny of tributary-spawning hatchery adults and, in some years, unmarked hatchery presmolts released into tributaries. Therefore, total spawner estimates (S) included the total number of hatchery adults released for natural spawning and the hatchery adults that contributed to the unmarked presmolt releases:

$$(2) \qquad S = S_w + \frac{(H_a + H_p)}{S_{ps}}$$

where H_a is the number of hatchery adults released into streams to spawn naturally, H_p is the number of hatchery adults contributing to the unmarked presmolt release, and S_{ps} is prespawning survival rate (=0.8).

Numbers of hatchery adults contributing to the unmarked presmolt releases (H_p) were estimated based on hatchery-specific average fecundity, a 1:1 sex ratio at spawning, and 0.9 survival rate from egg to presmolt release (Kiefer et al. 1991; Olsen et al. 1991; D. Cannamela, Idaho Department of Fish and Game, Boise, Idaho, personal communication). Unmarked hatchery-origin smolts contributed a small proportion (<10%) to the wild smolt estimate before brood year 1990. In recent brood years, 1990–1997, H_p was zero because the released presmolts were marked (or otherwise identified) and counted in the hatchery smolt estimate rather than in the wild smolt estimate. The survival rate of released hatchery chinook fry to the parr stage was comparable with that of wild chinook (15%) for selected Idaho streams (Scully et al. 1990); the H_p estimate is based on an assumption that parr-to-smolt survival is also comparable between hatchery and wild fish. Numbers of hatchery adults (H_a) and adults contributing to unmarked presmolt releases (H_p) are shown in Table 1.

Wild smolt (progeny) estimates

Numbers of wild spring and summer chinook smolts are also estimated at the uppermost dam (Fig. 1). However, in contrast with the adult spawner time series, there is not one continuous time series of estimates for wild smolt numbers. Therefore, we relied on

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two sets of smolt data that encompass different segments of time. Estimates of wild smolts were available for smolt years 1964–1984 (brood year + 2) from marking studies described in Raymond (1979, 1988). There was no way to estimate wild smolts for smolt years 1985–1991, and consequently, those years could not be included in this analysis. Estimates of wild smolts for smolt years 1992–1999 were available based on fish passage indices from the Fish Passage Center (1999).

Raymond (1979, 1988) estimated numbers of wild stream-type chinook smolts arriving at the uppermost dam, 1964–1984, using marked groups and estimates of collection efficiency at the dam. Raymond (1979) derived the proportions of wild and hatchery smolts from survival rate calculations on wild fish marked and released in the Salmon River, Idaho, and hatchery fish marked and released from Rapid River Hatchery on the Salmon River (the only hatchery releasing substantial numbers in these years). Raymond (1988) updated his earlier published estimates of numbers of wild and hatchery stream-type chinook smolts arriving at Lower Granite Dam using the same methods as in the 1979 paper. The number of hatchery smolts each year was derived from the total numbers released and their survival rate to the first dam; this number, when subtracted from the total population estimate calculated at the first dam, provided an estimate of wild smolts each year. For most years, Raymond (1988) concluded that sufficient numbers of marked hatchery smolts were recovered for survival rate calculations. For the few years in which there was no marking at hatcheries, or recoveries were insufficient for the analysis, he used the average survival rate for years bracketing the missing data. Raymond (1988) did not specify the years to which he applied average survival rates; however, a review of Rapid River Hatchery annual reports (T. Elms-Cockrum, Idaho Department of Fish and Game, Boise, Idaho, personal communication) indicated no marking at this facility in smolt migration years 1976, 1978, and 1981.

Recent year (1992–1999) estimates of wild stream-type chinook smolts were derived from fish passage indices, which were calculated from smolt sampling programs at the uppermost dam (Fish Passage Center 1999). Wild smolts were identified at Lower Granite Dam by examination of fin clips in 1993–1999 (all hatchery fish were marked with adipose clips, ventral clips, and (or) tags) and by scale pattern analysis in 1992 (Borgeson and Bowden 1994). The passage indices represent a relative indicator of population abundance, computed by dividing the daily fish collection estimate at the dam by the proportion of flow passing through the sampled unit or powerhouse relative to river flow (Fish Passage Center 1999). There are four possible routes past the dams: (1) through turbines, (2) over the spillway, (3) guided by screens from turbine intakes, collected, and bypassed back to the river, or (4) guided, collected, and then transported in barges or trucks. Sampling programs enumerate smolts passing by routes 3 and 4. However, estimates of total smolts require estimates of the proportion of smolts guided away from the turbines (fish guidance efficiency (FGE)) and proportion of smolts passing via spill in relation to the proportion of river flow being spilled (spill efficiency). The daily passage indices account for daily changes in spill proportion under the assumption that the proportion of fish passing through spill will be close to the proportion of water being spilled (Fish Passage Center 1999) and do not account for the proportion of fish passing through turbines. We accounted for the total number of smolts passing through the dam by dividing the daily fish passage indices by an estimated FGE of 0.55 (Marmorek et al. 1998).

Annual productivity (ln(smolts/spawner))

The annual estimates for wild smolts and wild spawners were used to compute an annual freshwater productivity, expressed as ln(smolts/spawner). This is an annual productivity measured at the FSR life stage for the observed spawner numbers. We investigated evidence for density dependence in the relationship of ln(smolts/spawner) versus spawner by linear regression for the full time series of data and by analysis of covariance (ANCOVA) with spawner as the covariate and time period as the treatment (see below).

Smolt-to-adult return rates

Smolt-to-adult return rate (SAR) provides a measure of overall survival from the outmigrating smolt stage (after the FSR stage) to the returning adult recruit stage (Fig. 1). SARs were estimated for the aggregate population of Snake River wild spring and summer chinook, brood years 1962–1994. SAR was calculated as the total number of recruits (age 3 jacks, which are precocious males, and age 4–5 adults) returning to the Columbia River mouth by brood year divided by number of smolts from that brood arriving at the uppermost dam on the Snake River (Lower Granite Dam since 1975). We estimated SAR to the Columbia River mouth because that measure is consistent with the definition from the data set for adult survival rate indices (SRI-1; Schaller et al. 1999). We also updated the adult survival rate indices for brood years 1991–1994.

We estimated the number of wild adult recruits at the Columbia River mouth (Fig. 1) in order to have a consistent measure that accounts for variable harvest rates and adult upstream passage survival rates (Appendix). First, using the wild component of the uppermost dam count for return years 1962–1999 ($S_w + W_{bar}$ from eq. 1 above), we partitioned the total counts by age (Beamesderfer et al. 1997; C.E. Petrosky, unpublished data). Because recruits should include all fish (ages 3–5) and adult returns from dam counts represented age 4–5 fish only, we increased the adult returns to include the age 3 fish (jacks). In the next step, we calculated returns by age to the Columbia River mouth by dividing the age-structured return to the upper dam by the annual upstream passage survival rate and then dividing by the annual survival rate through the main stem fisheries (Beamesderfer et al. 1997). In the final step, we allocated the age-structured returns to the Columbia River mouth to the appropriate smolt year (brood year + 2). We used the natural log transformation of SAR in analyses (Peterman 1981).

Productivity and survival rate analysis

We compared spawners, smolts, ln(smolts/spawner), and ln(SAR) before and after the completion of the hydrosystem using analysis of variance (ANOVA). Periods were defined as brood years 1962–1974 for Period 1 and brood years 1975–1997 for Period 2, where data were available.

Productivity and survival rate indices at the FSR life stage were estimated for the two periods for the Snake River spring and summer chinook aggregate population. Productivity is defined here as the natural log of the ratio of progeny (smolts) to parents (spawners) in the absence of density-dependent mortality. Spawner and smolt data, at the FSR life stage, can be fit to the Ricker (1975) parent–progeny function

$$(3) \qquad R_{\text{smolts}} = e^{a} S e^{-\beta S}$$

where R_{smolts} is smolts measured at the uppermost dam and S is spawners. The a and β parameters are estimated by the log transformation of eq. 1, and productivity is measured as the intercept, or Ricker a.

Parent–progeny analysis normally assumes that the average parent–progeny relationship does not change over time. Often the parent–progeny curve will indeed change (nonstationarity) due to physical change in the environment or change in stock structure (Walters 1987; Hilborn and Walters 1992). We tested for evidence of nonstationarity in the parent–progeny functions caused by physical change in the FSR habitat or other influence early in the life cycle. Large changes in density-independent mortality (caused by change in the FSR stage physical environment) within a time series would cause nonstationary behavior in the parent–progeny function. We used two methods, suggested by Walters (1987), to detect

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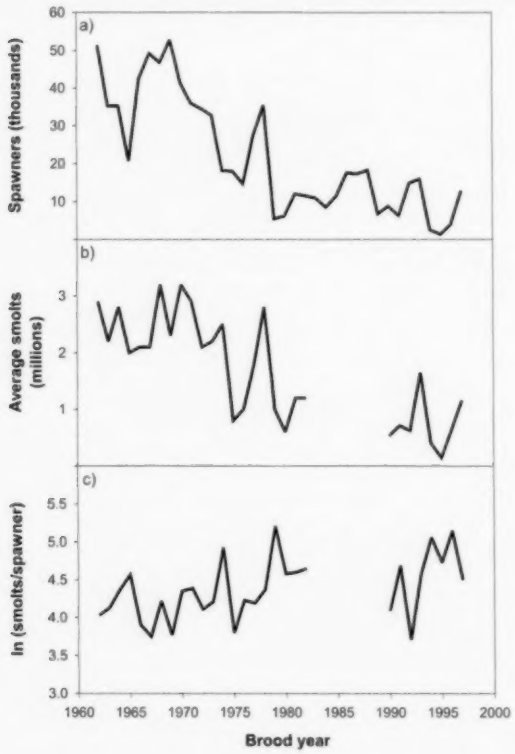
Fig. 2. (*a*) Spawners, (*b*) average smolts, and (*c*) annual FSR productivity (ln(smolts/spawner)) for brood years 1962–1997.



if a systematic change in the parent–progeny relationships took place associated with a change in the physical environment: (*i*) covariance analysis of data in time blocks associated with the physical change and (*ii*) evaluation of time series of parent–progeny model deviations.

We evaluated whether productivity declined for the aggregate Snake River population from Period 1 to Period 2 at the FSR life stage. We used ANCOVA to examine differences between the two periods for the intercept (Ricker a value) of the relationship of $\ln(R_{\text{smolts}}/S)$ versus S. Then, we contrasted change in FSR productivity with productivity change estimated over the entire life cycle (adult recruits/spawner) (Schaller et al. 1999) to determine if the change at the FSR life stage could explain the overall productivity change:

$$(4) \qquad \ln(R_{\text{smolts}_{i,j}}/S_{i,j}) = \tau_i + a - \beta(S_{i,j} - \bar{S}_{\cdot}) + \varepsilon_{i,j}$$

where τ_i is the class effect (period), a is the overall intercept, β is the overall slope, $\varepsilon_{i,j}$ is the normally distributed residual, i is the class (period), and j is the observation.

The assumption of homogeneity of slopes was first tested based on significant interaction between treatment (period) and the covariate (spawners). Then ANCOVA was run to estimate the period effect on $\ln(R_{\text{smolts}}/S)$, taking into account spawning level (covariate). The measure of productivity by period was estimated using $\tau_i + a$ from the ANCOVA results (eq. 4). This is equivalent to the Ricker a parameter by period assuming a common slope (β) over periods. The ANCOVA also tests for density dependence as indicated by a significant negative slope for the relationship between smolts per spawner and spawners. Tests were conducted at the $\alpha = 0.05$ level.

Survival rate indices provide a time series of density-independent survival estimates through deviations of observed $R_{\text{progeny}}/S_{\text{parent}}$ from those predicted by the fitted parent–progeny function for a specified time period. The deviations, or survival rate indices at the FSR life stage, were expressed as ln[(observed R_{smolts}/S)/(predicted R_{smolts}/S)], where predicted values were based on fits to data from different time periods. The natural log of these ratios transform the differences, such that they tend to be normally distributed (Peterman 1981). This approach, using the residuals as an index of FSR survival rate, was used to be consistent with the method used by Schaller et al. (1999) for measuring survival over the entire life cycle and is similar to the approaches in Hilborn and Walters (1992, fig. 7.2.1), Myers et al. (1997), and Peterman et al. (1998). The FSR survival rate index represented the deviation of the Period 2 observations from Period 1 predicted values based on the ANCOVA fit of eq. 4. We evaluated if there was a change in FSR survival rate index between Period 1 and Period 2 using ANOVA and contrasted the time series of survival rates at the FSR stage with those for the entire life cycle.

We investigated the power and effect sizes for the ANCOVA tests of significance for the period effect on FSR productivity. Peterman (1990) noted that in fisheries applications, a large cost could be associated with falsely assuming that there is no effect (type II error) and argued the need to use information on power and minimum detectable differences. It is possible that a real difference in FSR productivity may exist, but no significant difference would be found because of large variability or small sample size. From a management perspective, our interest was threefold. First, how likely were we to detect a true difference in the FSR productivity at the magnitude estimated from the smolt/spawner analysis? Second, what magnitude of change in the FSR productivity would have been necessary in order to have sufficient power to measure the change, given the data? Finally, if we observed a change in FSR productivity similar to the change of the magnitude exhibited over the entire life cycle (Schaller et al. 1999), would there be sufficient power to be confident that we detected a true change at the FSR stage?

The power of the ANCOVA was estimated using the SAS power macro (Latour 2000) as

$$(5) \qquad \text{Power} = 1 - F\{F_{\text{crit}}, \text{df}_{\text{hyp}}, n - \text{df}_{\text{mod}} - 1, \lambda\}$$

and delta, the effect size, is

$$(6) \qquad \delta^2 = \frac{\text{SS}_{\text{hyp}}}{n}$$

where

$$(7) \qquad \lambda = \frac{n\delta^2}{\sigma^2} = \frac{\text{SS}_{\text{hyp}}}{\sigma^2} \approx \frac{\text{SS}_{\text{hyp}}}{\text{MSE}}.$$

We first estimated the power of the period effect for ANCOVA for FSR productivity and used that data set, with its associated variance and sample size, to generate a relationship between power and the difference in period effects between Period 1 and Period 2 ($\tau_1 - \tau_2$) for FSR productivity. The $\tau_1 - \tau_2$ can then be converted directly to the change in productivity for smolts per spawner ($= 1 - e^{-(\tau_1 - \tau_2)}$). We then compared the change in overall productivity estimated from the adult recruits/spawner versus spawner in Schaller et al. (1999) with the change in FSR productivity from this data set. This comparison allowed us to determine how much power we would have had for detecting the difference in overall productivity between periods (e.g., before and after the hydrosystem). The sensitivity of the productivity analyses to alternative estimates for smolts (using the PATH set of parameter val-

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Table 1. Snake River spring and summer chinook dam counts (U_{count}), hatchery returns (R_{rtn}), wild and hatchery harvest, wild spawners (S_w), hatchery adults released for spawning (H_a), hatchery adults used in presmolt releases (H_p), and total spawners (S).

Brood year	Upper dam U_{count}	R_{rtn}	Upper dam wild	Proportion wild in harvest	Harvest wild plus hatchery	S_w	H_a	H_p	S
1962	64 252		64 252	1.00	12 816	51 436			51 436
1963	47 653		47 653	1.00	12 390	35 263			35 263
1964	44 700	763	43 746	1.00	8 465	35 281			35 281
1965	21 900	649	21 089	1.00	414	20 675			20 675
1966	54 500	1 789	52 264	1.00	9 744	42 520			42 520
1967	57 700	1 083	56 143	0.98	7 057	49 249			49 249
1968	63 000	4 390	57 125	0.97	10 599	46 836			46 836
1969	68 300	2 817	64 089	0.95	12 198	52 443	324	12	52 863
1970	54 400	6 476	45 734	0.92	5 803	40 388	358	438	41 383
1971	43 200	3 357	38 308	0.85	3 657	35 208	119	533	36 023
1972	57 100	12 310	38 073	0.56	6 667	34 318	152		34 508
1973	63 700	17 099	38 788	0.71	9 859	31 760	369	428	32 756
1974	23 100	3 494	18 344	0.79	1 500	17 155	67	721	18 140
1975	23 700	4 739	17 776	0.00		17 776		167	17 985
1976	22 900	6 734	14 483	0.00		14 483		106	14 615
1977	43 900	10 460	29 128	0.61	3 500	26 986	24	238	27 313
1978	52 300	7 735	40 978	0.81	7 000	35 301		124	35 455
1979	9 551	3 438	5 253	0.00		5 253	32	153	5 484
1980	8 148	1 612	6 133	0.00		6 133	33		6 174
1981	16 441	4 162	11 238	1.00	49	11 189	661		12 016
1982	16 577	4 228	11 292	1.00	26	11 266	144	53	11 512
1983	13 402	2 749	9 965	1.00	31	9 934	271	544	10 953
1984	11 921	3 413	7 654	0.00		7 654	568	105	8 495
1985	30 269	12 942	8 822	0.00	4 216	8 822	1 104	1032	11 492
1986	37 876	17 985	11 288	0.00	3 287	11 286	4 319	689	17 546
1987	34 726	15 909	10 969	0.01	3 132	10 933	3 610	1450	17 258
1988	35 640	14 676	11 540	0.01	4 638	11 506	3 575	1832	18 264
1989	16 124	7 513	5 548	0.02	964	5 532	917	21	6 704
1990	22 408	9 812	6 926	0.01	2 591	6 909	1 475	31	8 791
1991	10 432	4 018	5 407	0.33	3	5 406	598	59	6 227
1992	24 405	8 927	11 465	0.02	1 458	11 432	2 452	274	14 839
1993	28 924	13 110	10 824	0.07	1 468	10 726	3 414	757	15 940
1994	3 915	1 749	1 721	0.25	8	1 719	595		2 463
1995	1 799	510	1 162	1.00	2	1 160	113		1 301
1996	6 823	2 685	3 474	0.00	40	3 474	367		3 932
1997	44 563	22 710	7 559	0.00	7 049	7 551	4 255		12 870
1998	14 242	3 566	8 337	0.05	1 263	8 269			8 269
1999	6 743	2 623	3 432	0.00	133	3 432			3 432
Average	31 611	6 375	22 315	0.47	3 738	19 649	787	257	20 954
Minimum	1 799		1 162	0.00		1 160			1 301
Maximum	68 300	22 710	64 252	1.00	12 816	52 443	4 319	1832	52 863

ues for FGE and spill efficiency) and adults (using wild-origin spawners, S_w) was evaluated.

Results

Wild spawner estimates

The estimated number of spring and summer chinook spawners (S) averaged 20 954 (range 1301 – 52 863) for brood years 1962–1999 (Fig. 2*a*; Table 1). Wild-origin adults (S_w) made up from 59 to 100% of the spawners. Spawner numbers dropped steadily in the early 1970s and remained at low levels through the most recent years (Fig. 2*a*). Spawners in Period 2, after the completion of the hydrosystem, were significantly lower than those in Period 1, before the completion of the hydrosystem ($F = 67.53$, $P = 0.0001$).

Wild smolt estimates

Wild smolt estimates at the upper dam averaged 1 680 000 for the time series with a range of 147 000 – 3 200 000 (Table 2; Fig. 2*b*). Smolt numbers demonstrated a period of high variability in the 1970s followed by very low numbers in the early 1980s and recent years. Similar to spawner estimates, smolt numbers were significantly lower in the period after the development of the hydrosystem was completed

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Table 2. Estimated number of wild chinook smolts passing the uppermost dam, smolts per spawner, and ln(smolts/spawner).

Brood year	Smolt year	Wild smolts	Smolts per spawner	ln(smolts/spawner)
1962	1964	2 900 000	56	4.03
1963	1965	2 200 000	62	4.13
1964	1966	2 800 000	79	4.37
1965	1967	2 000 000	97	4.57
1966	1968	2 100 000	49	3.90
1967	1969	2 100 000	43	3.75
1968	1970	3 200 000	68	4.22
1969	1971	2 300 000	44	3.77
1970	1972	3 200 000	77	4.35
1971	1973	2 900 000	81	4.39
1972	1974	2 100 000	61	4.11
1973	1975	2 200 000	67	4.21
1974	1976	2 500 000	138	4.93
1975	1977	800 000	44	3.80
1976	1978	1 000 000	68	4.23
1977	1979	1 800 000	66	4.19
1978	1980	2 800 000	79	4.37
1979	1981	1 000 000	182	5.21
1980	1982	600 000	97	4.58
1981	1983	1 200 000	100	4.60
1982	1984	1 200 000	104	4.65
1983	1985	na	na	na
1984	1986	na	na	na
1985	1987	na	na	na
1986	1988	na	na	na
1987	1989	na	na	na
1988	1990	na	na	na
1989	1991	na	na	na
1990	1992	527 424	60	4.09
1991	1993	680 793	109	4.69
1992	1994	604 313	41	3.71
1993	1995	1 577 215	99	4.59
1994	1996	389 376	158	5.06
1995	1997	146 873	113	4.73
1996	1998	680 989	173	5.15
1997	1999	1 156 931	90	4.50
Average		1 678 066	86	4.38
Range		146 873 – 3 020 000	41–182	(3.71–5.21)

($F = 23.44$, $P = 0.0001$), although no data were available for a few years in Period 2.

Annual productivity

Snake River spring and summer chinook produced an average of 86 smolts per spawner for the time series (Table 2). The ln(smolts/spawner) estimates averaged 4.38 (Table 2). Annual FSR productivity, ln(smolts/spawner), showed a weak (insignificant) increasing trend through the time series (Fig. 2*c*) ($F = 4.18$, $P = 0.0508$). The increasing trend in ln(smolts/spawner) combined with a decreasing trend in spawner numbers (Figs. 2*a* and 2*c*) is consistent with density dependence in the FSR life stage. Regression of ln(smolts/spawner) versus spawner for the entire time series (Fig. 3) had a significant negative slope ($F = 20.62$, $P = 0.0001$).

Smolt-to-adult return rates

SARs of Snake River wild spring and summer chinook declined sharply after the 1960s. Mean SARs were 5.2% in Period 1 and 1.2% in Period 2 (Fig. 4; Appendix). ln(SAR) values were significantly lower in Period 2 (posthydrosystem) compared with Period 1 (prehydrosystem completion) ($F = 12.93$, $P = 0.0015$). The SARs estimated here differ from those reported in Raymond (1988) because we recalculated and updated wild recruits and incorporated adult upstream passage survival (which was not incorporated in Raymond's (1988) SAR estimates; Fig. 1). The pattern of declining SARs after the completion of the hydrosystem, however, is the same for both estimates.

Survival of smolts entering the hydrosystem to adults returning to the Columbia River mouth (SAR) demonstrated a major decline (Fig. 4). This decline was not observed in the annual pattern of smolts per spawner through the FSR life stage. Whereas ln(SAR) declined from –3.5 to –4.5 from Period 1 to Period 2, ln(smolts/spawner) increased slightly from 4.2 to 4.5. The relationship between smolts and spawn-

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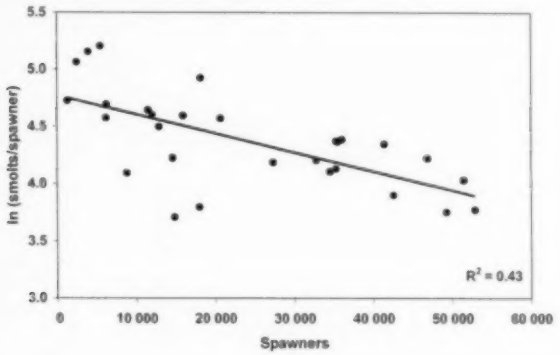
Fig. 3. Relationship between annual FSR productivity (ln(smolts/spawner)) and spawners (S) for the time series of brood years 1962–1997.



ers exhibited density dependence (Fig. 3). Hence, we would expect ln(smolts/spawner) to increase as spawner numbers decreased, if average FSR conditions had indeed remained stationary through the time series.

Productivity and survival rate analysis

The FSR productivity showed no significant decline since completion of the hydropower system. The period effect was not significant in the ANCOVA tests (P = 0.058); however, the point estimate for the period effect suggested a decrease in FSR productivity of –0.37 (insignificant). Results were also insensitive to alternative PATH parameter values of FGE and spill efficiency used for smolt estimates (Marmorek et al. 1998) and to the use of only wild-origin spawners (S_w) in the ANCOVA. Period effects were insignificant for all alternative assumptions (P range 0.059–0.154) in this sensitivity analysis.

The ANCOVA test demonstrated a strong pattern of density dependence (decreasing smolts per spawner at high spawner levels), as demonstrated by the slope parameter (β) in the ANCOVA. The slope was negative and highly significant (β = –0.000026, P = 0.0001). The assumption of a common slope between time periods (homogeneity of β) was plausible based on an insignificant test (P = 0.96) for interaction of period effect (treatment) and spawners (covariate).

We compared an overall survival rate index (updated SRI-1 from Schaller et al. 1999), which represents deviations in survival rate across the salmon life cycle, with the FSR survival rate index, which represents deviations in survival rate for the egg-to-smolt FSR stage alone. The overall survival rate index across the life cycle showed a steep decline that was significantly lower after the completion of the hydrosystem as compared with before the hydrosystem was completed (Fig. 5) (F = 9.87, P = 0.0042). The pattern of this overall survival rate index also indicated greater variability in Period 2 (Schaller et al. 1999). In contrast, the pattern from the FSR index showed less of a downward trend through time and was considerably less variable in Period 2 compared with the overall survival rate index (Fig. 5). However, there was a significant difference in the FSR survival rate index between the two periods, before and after the completion of the hydrosystem (F = 10.84, P = 0.0029). For the FSR index, three of the largest negative residuals (poor survival) occurred in Period 2 and were associated with major drought years during the year of smolt migration (1977, 1992, and 1994). Overall survival based on the survival rate index was also very low following smolt years 1977 and 1992.

The change in productivity measured by τ represents the average change in density-independent survival rates between periods. Power analysis indicates that most of the productivity decline, from period 1 to period 2, over the entire life cycle cannot be explained by decreases in FSR productivity but that comparatively small changes cannot be ruled out. The FSR productivity showed no significant decline since completion of the hydrosystem; however, the power to detect a true difference of this magnitude was moderately low (power = 0.47) (Fig. 6). This power estimate corresponds to a change in period effect ($\tau_1 - \tau_2$) of 0.37 or a drop in productivity of 31% ($1 - e^{-(\tau_1 - \tau_2)} = 0.31$). However, for this data set, if we had observed a change in period effect of 0.7 or greater, or a drop in productivity of 50% or greater, we would have had >80% power to detect a change, given the observed variance and sample size in the FSR data set. In contrast with the FSR stage, overall productivity for adult recruits/spawner dropped by 86% averaged across the seven indicator stocks ($\tau_1 - \tau_2$ = 1.96; see Schaller et al. 1999). That magnitude of change would have resulted in almost 100% power to detect a change, or period effect, in the FSR data set (Fig. 6). This comparison indicates that if the FSR productivity had declined by 86%, as observed for adult recruit/spawner, we would have had very high power to detect that type of change in the FSR life stage.

Discussion

Snake River stream-type chinook have declined dramatically since completion of the hydrosystem and have been listed as threatened under the Endangered Species Act since 1992. Declines in life cycle productivity and survival rates for Snake River stocks were greater than for similar stocks, which migrate past fewer dams (Schaller et al. 1999). In this paper, we tested the hypothesis that a decline in productivity and survival rate in the FSR stage since completion of the hydrosystem was at a magnitude that might explain the declines observed over the entire life cycle.

We found little to no empirical support for a marked decline in FSR productivity and survival rate since completion of the hydrosystem of the magnitude observed over the entire life cycle (Schaller et al. 1999). However, we cannot rule out comparatively minor decreases in FSR productivity and survival rate since the hydrosystem was completed. Estimated change in FSR productivity between pre- and post-hydrosystem completion was insignificant and small compared with the overall productivity decline. FSR productivity and survival rate change was insensitive to uncertainty in the smolt and spawner estimates. We found strong evidence for density dependence at the FSR stage consistent with observations of density dependence over the entire life cycle (Schaller et al. 1999).

Power was moderately low for detecting change of the estimated magnitude for FSR productivity but clearly would be sufficient to detect the magnitude of change observed over the entire life cycle, had the overall decline occurred

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Fig. 4. SAR (bars) and smolts per spawner (line) for those years in the time series where data were available. The SAR describes survival during mainstem downstream migration back to returning adults, whereas the number of smolts per spawner describes freshwater productivity in upstream FSR areas.

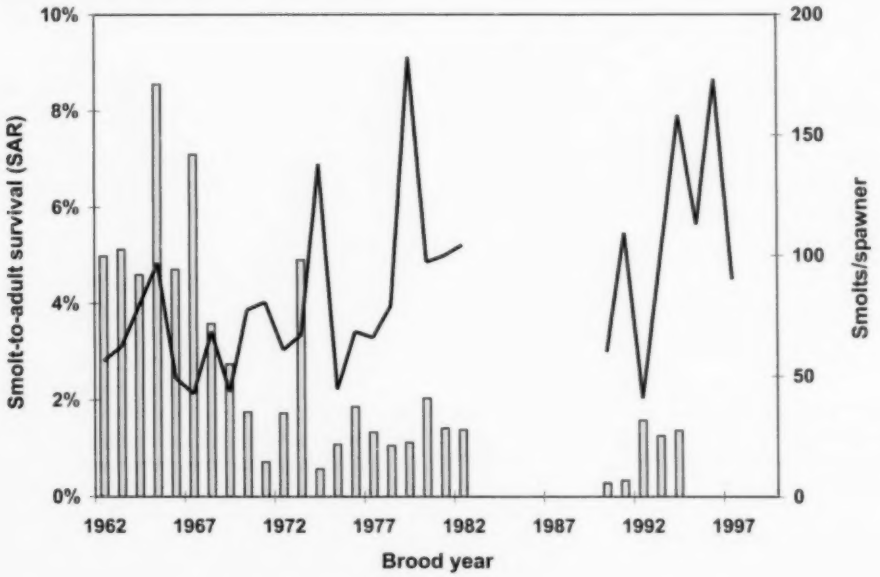
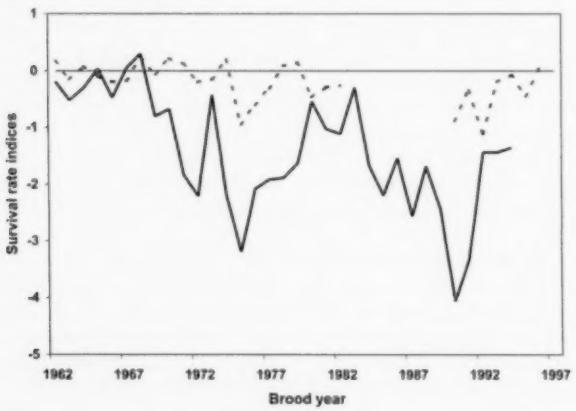


Fig. 5. Survival rate indices calculated over the entire life cycle from adult spawner and recruit data (solid line) and from the FSR stage (broken line). The overall survival rate index (updated SRI-1 from Schaller et al. 1999) represents deviations in survival rate across the salmon life cycle. The FSR survival rate index (ln(smolts/spawner)) represents deviations in survival rate for the egg-to-smolt FSR stage alone.



primarily in the FSR stage. In order to have a statistically significant result for change in FSR productivity between periods, we would have needed to observe a drop of 50% in smolts per spawner (at low spawner density). In comparison, productivity estimated from adult recruit/spawner information declined 86% over the same period. Although we could not rule out small changes in FSR productivity and observed small changes in FSR survival rates relative to the overall decline, this relatively small decrease is not trivial (approximately 30% in smolts per spawner and in survival rate). The decrease was presumably due to some combination of FSR habitat change, drought, or other climate condition, hatchery effect, or depensatory mechanism affecting the aggregate population.

The trends and patterns in FSR productivity and survival rate observed for aggregate populations may not extend to individual populations within the Snake River Basin. Although poorly quantified, dynamics of individual spawning populations at the FSR life stage can be expected to respond to habitat conditions at the local and basin scales. A broad mix of land use influences, from minor (wilderness) to management for irrigated agriculture, livestock grazing, logging, and mining, existed throughout the time series (Fulton 1968; Beamesderfer et al. 1997). Negative trends in habitat condition (quality pools) are evident in several managed watersheds, whereas streams in wilderness or unroaded watersheds have shown greater stability over half-century time scales (McIntosh et al. 1994). Reductions in sediment deposition have also been documented in the heavily degraded South Fork Salmon River since the mid-1960s (Platts et al. 1989), and major fish screening programs were completed by the late 1960s in the upper Salmon and Grand Ronde rivers. While FSR productivity and survival rate of individual populations would be expected to track with these localized trends, the aggregate data provide no evidence for a major shift in the FSR stage since completion of the hydrosystem. However, this analysis did not address whether there was a significant decline in FSR productivity and survival rate prior to the completion of the hydrosystem because the smolt and spawner data were not collected prior to the 1960s.

In contrast with the relative stationarity in FSR productivity and survival rates since hydrosystem completion, significant survival decreases were evident in the smolt-to-adult

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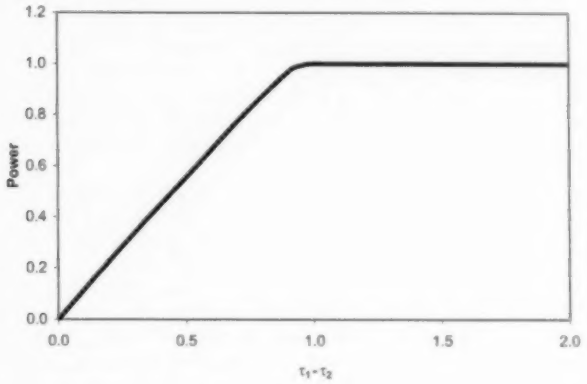
Fig. 6. Relationship between statistical power and the difference in productivity between periods for the ANCOVA ln(smolts/spawner) versus spawner with periods before and after the completion of the hydrosystem (Period 1, 1962–1974; Period 2, 1975–1997).



life stage. This life stage includes most of the life cycle except for the FSR stage, from the time shortly after smolts enter the hydrosystem to adults returning to the Columbia River mouth. The magnitude of change in ln(SAR) (–1.0, a 63% drop in survival rate) could explain most of the drop in productivity estimated for the entire life cycle (Schaller et al. 1999). Decreases in SAR are most likely due to factors outside the FSR life stage, such as degradation of migratory habitat through construction and operation of the hydrosystem, increased hatchery production, and changing estuary and ocean conditions.

Schaller et al. (1999) concluded that factors other than hydropower development have not played a significant role in the differential decline in performance between upriver and downriver stocks. The Snake River stocks above eight dams survived one third as well as downriver stocks migrating through three dams (Schaller et al. 1999; Deriso 2001) for this time period, after taking into account factors common to both groups. The additional decline in productivity of upriver stocks relative to downriver stocks indicates that this portion of the mortality is related to factors unique to upriver stocks. Patterns of Pacific Decadal Oscillation and salmon production would indicate that poor ocean conditions existed for Columbia River salmon after the late 1970s (Hare et al. 1999). However, the natural fluctuations in ocean productivity affecting all Columbia River stocks, in combination with mortality as a result of the hydrosystem, appear to have caused the severe declines in productivity and survival rates for the Snake River stocks. Temporal and spatial patterns of hatchery release numbers did not coincide with the differential changes in survival rates between upriver and downriver stocks (Schaller et al. 1999). Harvest rates were drastically reduced, in the early 1970s, in response to declines in upriver stream-type chinook abundance. Given that FSR life stage survival rate changes cannot explain the decreases in SAR or overall survival rates for Snake River stocks, it appears that the altered migration corridor has had a strong influence on the mortality that causes these differences in stock performance.

The analysis discussed here has important management implications for the recovery of Snake River stocks in relation to the decision of whether or not to remove the four lower Snake River dams. Recently, federal agencies have indicated that hydrosystem impacts may be compensated through off-site mitigation measures, which might improve habitat or change hatchery practices (National Marine Fisheries Service 2000). Based on this premise, some federal agencies believe that dam removal will therefore be unnecessary. For off-site mitigation through habitat improvements to offset hydrosystem impacts, life cycle survival rates of these stocks would have to increase nearly threefold overall (Peters and Marmorek 2000). The absence of major declines in FSR productivity and survival rates, combined with significant declines in SAR after hydrosystem completion, indicates that it is unlikely that we will be able to increase overall survival to a level sufficient for recovery based on improvements to FSR habitat. In addition, overall productivity and survival rate declines were common to Snake River index stocks in wilderness areas (Sulphur Creek, Minam River) and those with varying degrees of habitat degradation (Schaller et al. 1999). Past attempts to mitigate for hydrosystem impacts through hatcheries have also failed to recover the natural populations (Lichatowich 1999).

These examples demonstrate the importance of identifying the factor that is limiting the population for management decisions regarding the recovery of an endangered species. For some stocks, improvements to habitat would likely improve survival in the spawning and rearing stage. However, the expected improvements in survival in that life stage are unlikely to offset the impacts of the hydrosystem and increase survival overall to a level that ensures the recovery of Snake River spring and summer chinook.

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Appendix

Table A1. Smolt-to-adult survival rates (SAR) and adult return data required to estimate SAR.

Return year	Age 3 returns	Age 4 returns	Age 5 returns	Upstream passage survival rate	Mainstem harvest rate	Brood year recruits (ages 3–5)	SAR (%)
1962	3969	29 436	34 816	0.61	0.44	144 385	4.98
1963	8704	17 401	30 252	0.62	0.49	112 629	5.12
1964	4246	26 427	17 319	0.59	0.38	128 510	4.59
1965	4320	12 044	9 044	0.43	0.40	171 030	8.55
1966	6789	28 006	24 258	0.63	0.21	98 776	4.70
1967	6543	23 339	32 804	0.68	0.33	148 902	7.09
1968	6296	30 574	26 551	0.73	0.24	114 482	3.58
1969	4790	41 965	22 123	0.49	0.24	63 224	2.75
1970	2836	24 017	21 717	0.65	0.29	55 819	1.74
1971	4500	26 116	12 192	0.50	0.26	20 730	0.71
1972	1519	15 646	22 427	0.46	0.32	36 075	1.72
1973	746	15 461	23 327	0.66	0.32	107 870	4.90
1974	562	9 566	8 778	0.43	0.13	14 277	0.57
1975	152	3 847	13 929	0.49	0.00	8 623	1.08
1976	1230	8 697	5 785	0.51	0.00	18 598	1.86
1977	899	19 082	10 046	0.65	0.17	23 882	1.33
1978	15	3 562	37 416	0.56	0.04	29 511	1.05
1979	401	2 025	3 228	0.57	0.04	11 144	1.11
1980	442	3 868	2 265	0.48	0.04	12 201	2.03
1981	606	6 169	5 070	0.57	0.06	16 946	1.41
1982	628	6 077	5 215	0.50	0.07	16 539	1.38
1983	253	1 965	8 000	0.56	0.06	28 100	na
1984	421	3 927	3 727	0.66	0.06	8 355	na
1985	298	5 046	3 776	0.75	0.06	9 918	na
1986	555	5 098	6 190	0.72	0.06	14 033	na
1987	184	5 715	5 254	0.65	0.07	10 421	na
1988	200	1 687	9 853	0.60	0.09	22 991	na
1989	371	2 786	2 762	0.58	0.05	4 633	na
1990	80	4 045	2 881	0.68	0.06	1 475	0.28
1991	286	1 517	3 890	0.59	0.05	2 276	0.33
1992	346	6 704	4 761	0.66	0.04	9 502	1.57
1993	109	1 696	9 128	0.81	0.05	19 787	1.25
1994	13	332	1 389	0.78	0.05	5 305	1.36
1995	109	621	541	0.64	0.04		
1996	215	2 784	689	0.58	0.04		
1997	136	4 957	2 602	0.63	0.04		
1998	198	1 295	7 042	0.65	0.04		
1999	398	1 866	1 566	0.53	0.03		
Average							2.58
Minimum							0.28
Maximum							8.55

Note: Upstream passage survival rates and mainstem harvest rates are from Beamesderfer et al. (1997).

Appendix reference

Beamesderfer, R.C.P., Schaller, H.A., Zimmerman, M.P., Petrosky, C.E., Langness, O.P., and LaVoy, L. 1997. Spawner–recruit data for spring and summer chinook salmon populations in Idaho, Oregon, and Washington. *In* PATH — Plan for Analyzing and Testing Hypotheses: report of retrospective analysis for fiscal year 1997. *Compiled and edited by* D.R. Marmorek and C. Peters. ESSA Technologies Ltd., Vancouver, B.C.

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Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie

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Abstract: *Microcystis aeruginosa*, a planktonic colonial cyanobacterium, was not abundant in the 2-year period before zebra mussel (*Dreissena polymorpha*) establishment in Saginaw Bay (Lake Huron) but became abundant in three of five summers subsequent of mussel establishment. Using novel methods, we determined clearance, capture, and assimilation rates for zebra mussels feeding on natural and laboratory *M. aeruginosa* strains offered alone or in combination with other algae. Results were consistent with the hypothesis that zebra mussels promoted blooms of toxic *M. aeruginosa* in Saginaw Bay, western Lake Erie, and other lakes through selective rejection in pseudofeces. Mussels exhibited high feeding rates similar to those seen for a highly desirable food alga (*Cryptomonas*) with both large (>53 μm) and small (<53 μm) colonies of a nontoxic and a toxic laboratory strain of *M. aeruginosa* known to cause blockage of feeding in zooplankton. In experiments with naturally occurring toxic *M. aeruginosa* from Saginaw Bay and Lake Erie and a toxic isolate from Lake Erie, mussels exhibited lowered or normal filtering rates with rejection of *M. aeruginosa* in pseudofeces. Selective rejection depended on "unpalatable" toxic strains of *M. aeruginosa* occurring as large colonies that could be rejected efficiently while small desirable algae were ingested.

Résumé : *Mycrocystis aeruginosa*, une cyanobactérie qui forme des colonies planctoniques, s'est multipliée dans la Baie de Saginaw (Lac Huron) durant trois des cinq étés qui ont suivi l'établissement de la Moule zébrée (*Dreissena polymorpha*), alors qu'elle n'était pas abondante durant les deux années qui ont précédé cet établissement. Des méthodes inédites ont permis de déterminer les taux de clearance, de capture et d'assimilation de Moules zébrées alimentées de souches naturelles et de souches de laboratoire de *M. aeruginosa*, présentées seules ou en combinaison avec d'autres algues. Nos résultats s'accordent avec l'hypothèse qui veut que les Moules zébrées favorisent, par des rejets sélectifs dans leurs pseudofèces, la formation de fleurs d'eau à *M. aeruginosa* de souche toxique dans la baie de Saginaw, dans la région occidentale du lac Érié et dans d'autres lacs. Nourries de grandes (>53 μm) et de petites (<53 μm) colonies d'une souche non-toxique et d'une souche toxique de laboratoire de *M. aeruginosa*, qui inhibent l'alimentation chez le zooplancton, les moules maintiennent des taux élevés d'alimentation, semblables à ceux que l'on observe lorsqu'on les nourrit d'une algue très recherchée (*Cryptomonas*). Dans des expériences d'alimentation utilisant des souches naturelles de *M. aeruginosa* toxiques de la baie de Saginaw et du lac Érié et un isolat toxique du lac Érié, les moules présentent des taux de filtration réduits ou normaux et rejettent *M. aeruginosa* dans leurs pseudofèces. Ce rejet sélectif dépend de la présence de grandes colonies de souches toxiques «à goût désagréable» de *M. aeruginosa* qui peuvent être éliminées facilement, alors que les petites algues appétissantes sont ingérées.

[Traduit par la Rédaction]

Introduction

Noxious blooms of colonial cyanobacteria such as *Microcystis*, *Anabaena*, and *Aphanizomenon* are well known symptoms of eutrophication caused by excessive phosphorus loading (Smith 1983; Sommer et al. 1986). These blooms, which were common on Saginaw Bay (Stoermer and Theriot 1985; Bierman et al. 1984) and Lake Erie (Makarewicz 1993) during the 1960s and 1970s, diminished as phosphorus controls were instituted during the mid-1970s.

Microcystis and other cyanobacterial blooms may have serious consequences to aquatic ecosystem function and

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health, to aesthetics, and to wildlife and human health. *Microcystis* and some other cyanobacteria produce a potent class of hepatotoxins called microcystins that can poison aquatic organisms as well as wildlife, domestic animals, and humans that drink or ingest algae in the water (Carmichael 1996). The major exposure pathway of microcystin is through ingestion, although some toxins can be released upon death of the cells (Lampert 1982; Nizan et al. 1986; Fulton and Paerl 1987). The toxicity and large colonial size of *Microcystis* and other nuisance cyanobacteria can lower ingestion and assimilation rates of zooplankton (Lampert 1982; Nizan et al. 1986). Toxicity, lowered assimilation rates, and low nutritional quality of *Microcystis* can cause decreased survival and reproduction of zooplankton, thus leading to inefficient pelagic food webs (Fulton and Paerl 1987; Vanderploeg et al. 1996).

After zebra mussels (*Dreissena polymorpha*) became established in Saginaw Bay, there were anecdotal reports of *Microcystis aeruginosa* blooms on Saginaw Bay. *Microcystis* blooms were not expected on the bay because of decreased phosphorus loading. Thus, we wondered if zebra mussels were somehow causing these blooms. This hypothesis seemed plausible, as *Microcystis* blooms were reported in other systems where zebra mussels became established. In September 1995, there was an intense bloom of *M. aeruginosa* that was visible from shore and satellite imagery as a surface scum that covered much of the western basin of Lake Erie (Budd et al. 2001). Cyanobacterial blooms had not been seen on Lake Erie since phosphorus loading reductions were instituted (Makarewicz 1993; Nicholls and Hopkins 1993). *Microcystis* blooms following zebra mussel invasions have also been reported for Gull Lake and Gun Lake in Michigan (S. Hamilton, Michigan State University, East Lansing, Mich., personal communication) and the Bay of Quinte (Lake Ontario) (K. Nicholls, Ontario Ministry of the Environment, Toronto, Ont., personal communication). Additionally, late-summer blooms of the colonial cyanobacterium *Aphanizomenon* in Oneida Lake followed the zebra mussel invasion (Horgan and Mills 1997). An exception to this pattern occurred in the Hudson River, where *Microcystis* and other cyanobacteria have virtually disappeared since the establishment of zebra mussels (Smith et al. 1998).

The potential connection between zebra mussels and *M. aeruginosa* has important water quality management implications; it appears that zebra mussels have reversed progress made by nutrient control programs by increasing the probability of a cyanobacterial bloom. For example, in Saginaw Bay (Bierman et al. 1984), Lake Erie (Makarewicz 1993), Gull Lake (Tessier and Lauff 1992), and the Bay of Quinte (K. Nicholls, Ontario Ministry of the Environment, Toronto, Ont., personal communication), blooms occurred after drastic reductions in phosphorus loading and improvements in water quality.

Our preliminary observations involving traditional bottle experiments and direct observations with video during September 1994 showed that mussels did not ingest *M. aeruginosa* but nearly continually filtered and produced pseudofeces. This behavior led us to hypothesize that zebra mussels promoted and maintained *M. aeruginosa* blooms via rejection of *Microcystis* as pseudofeces. Fully stated, the hypothesis argued that (*i*) mussels would continually filter in the presence of *Microcystis*, (*ii*) mussels would ingest all algae except *Microcystis*, and (*iii*) mussels would produce loosely consolidated pseudofeces that would be injected back into the water column. If zebra mussel biomass and weight-specific filtering rate of the mussels allowed the mussels to clear the water at a rate representing a significant proportion of algal growth rate, then selective filtering would lead to *M. aeruginosa* dominance.

To investigate the selective feeding hypothesis, we initiated a series of experiments of *D. polymorpha* feeding on naturally occurring *M. aeruginosa* from Saginaw Bay and Lake Erie and on pure laboratory cultures of *M. aeruginosa*, including a strain isolated from the Lake Erie bloom of September 1995 (Brittain et al. 2000). In this paper, we examine how *M. aeruginosa* colony size, toxicity, and strain type affect mussel feeding behavior. Combining these experimental results with biomass of zebra mussels in Saginaw Bay and Lake Erie allowed us to quantify the magnitude of the selection process. Also, because *M. aeruginosa* blooms on Saginaw Bay were not documented, we examined time histories of phytoplankton composition before and after the zebra mussel invasion.

Materials and methods

Study sites and collections

Dreissena polymorpha and water from Saginaw Bay were collected from Station 5, a 3.5-m-deep station with a cobble, sand, and gravel substrate, in the inner bay (Nalepa et al. 1995) as part of a 6-year study of the impact of zebra mussels on the ecology of the bay. Temperature, chlorophyll *a* (Chl *a*), particulate organic carbon (POC), and algal composition were taken at this station at 1 m depth during May, September, and October in 1990 and at least monthly during 1991–1996. *Dreissena polymorpha* and water from western Lake Erie were collected during the *M. aeruginosa* bloom of September 1995 in Hatchery Bay of South Bass Island (40°40′01″N, 82°49′61″W). Generally, both inner Saginaw Bay (Nalepa et al. 1996) and western Lake Erie (Schertzer et al. 1987) are isothermal and well mixed. Western Lake Erie has a mean depth of 7.1 m, and inner Saginaw Bay has a mean depth of 5.1 m.

Dreissena polymorpha were hand collected by divers using SCUBA. Rocks with attached clusters of *D. polymorpha* were wrapped in moist paper towels and placed in coolers for transport. Mussels and water were kept cool and transported on the day of collection to the Great Lakes Environmental Research Laboratory (GLERL) and then kept at ambient temperature and photoperiod.

Reacclimation of mussels to natural seston containing *Microcystis*

A reacclimation period was provided to allow mussels to reestablish natural feeding behaviors for the ambient lake seston. Typically, mussels were collected in the late morning and used in experiments after ~17 h of reacclimation to lake seston at the ambient lake temperature. Upon arrival at GLERL within 4–5 h of collection, mussels typically ranging in length between 13 and 16 mm were gently removed from the rocks to which they were attached by cutting their byssal threads with a razorblade, and periphyton adhering to the shells was gently brushed off. Twenty to 30 of these mussels were placed in a 30-L aquarium filled with lake water collected that day. After ~14 h, all mussels were placed in another 30-L aquarium filled with new lake water for 3 h before being used in the feeding experiments.

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Chlorophyll, temperature, POC, and ash-free dry weight

For the time histories of temperature and triplicate measurements of Chl *a* and POC, methods of Nalepa et al. (1996) were used. For Chl *a* determinations for feeding experiments, the sequential filtration apparatus (Bowers 1980) fitted with a GF/F filter downstream of a 53-μm-mesh Nitex filter (cut from Nitex screen) was used to collect >53 and <53 μm size fractions. These filters were extracted in *N*,*N*-dimethylformamide and analyzed fluorometrically (Speziale et al. 1984). Precision ($SE/\bar{X}$) for all replicate Chl *a* analyses for monitoring and experiments was better than 5%.

Ash-free dry weights (AFDW) of mussels were determined by removing mussels from shells, drying for 2 days at 60°C, and ashing at 550°C for 1 h. In some experiments, AFDW was determined from length–AFDW regressions of mussels collected at the same station and time.

Algal culturing and phytoplankton analysis

Algae were harvested in exponential phase, except for *M. aeruginosa* strain CCAP 1450/11(described below), which was harvested in the early stationary phase of growth. The appropriate quantity of algal suspension was diluted into ambient seston or 0.2-μm-filtered lake water as required for the experiment. All culture media were filter sterilized with a 0.2-μm membrane filter. Cryptophytes were grown in WC media (Vanderploeg et al. 1996) modified by doubling $NaHCO_3$, halving $NaNO_3$, and adding 0.1 mM NH_4Cl at a light intensity of 40 μmol quanta·m^{-2}·s^{-1} on 16 h light : 8 h dark cycle at 20°C. All *M. aeruginosa* strains were cultured in BG-11_0 + $NaNO_3$ (2 mM) + $NaHCO_3$ (10 mM) medium (Rippka and Herdman 1992) plus the WC vitamin mixture under continuous light at 40 μmol quanta·m^{-2}·s^{-1} at 25°C.

Lake water samples for phytoplankton enumeration were preserved in 1% Lugol's solution, and 2- to 50-mL subsamples (depending on algal concentration) were filtered onto membrane filters for permanent mounting on slides; cell dimensions of the different taxa were converted to cell carbon for reporting biomass (Fahnenstiel et al. 1998). Some experimenters working with laboratory cultures reported algal concentrations in units of cells per litre. For comparison with our laboratory results, we converted them into volumetric units of cubic millimetres per litre from reported cell dimensions.

Toxin concentration

Microcystin, the toxin produced by *Microcystis*, was measured by enzyme-linked immunosorbent assay (ELISA) of polyclonal antibodies to microcystin-LR (An and Carmichael 1994). Seston or algal cultures were filtered onto GF/F filters and extracted in methanol. Typically, 1–2 L of seston and 25–50 mL of *Microcystis* culture were filtered, and triplicate ELISAs were run with a precision ($SE/\bar{X}$) of ~10%. Microcystin concentration was normalized to Chl *a* concentration and dry weight of the seston or *Microcystis* culture. To estimate microcystin concentration per unit of *Microcystis* Chl *a* in seston, we assumed that the proportion of *Microcystis* Chl *a* in the seston was the same as that for algal carbon estimated from algal counts.

General design of filtering experiments

To obtain estimates of filtering rates and other feeding rate variables on natural seston and algal cultures, we developed a novel experimental approach based on methods of Walz (1978) and Vanderploeg et al. (1995). Experiments were usually conducted in 2-L beakers in dim light (4–8 μmol quanta·m^{-2}·s^{-1}). Gentle bubbling provided agitation to assure mixing in four experimental (with mussels) and three control (without mussels) beakers and to keep particles suspended during the 1.5- to 3-h experiments. Usually, four mussels were placed in each experimental beaker. Enough phosphorus and nitrogen were added to the beakers to saturate nutrient uptake by the algae and to prevent different algal growth rates from occurring in the control versus experimental beakers as a result of mussel nutrient excretion; final concentrations were 12.5 and 250 μM phosphate and nitrate, respectively, or 2.5, 25, and 25 μM phosphate, nitrate, and ammonium.

We measured changes in Chl *a* concentration in small (<53 μm) and large (>53 μm) size fractions to examine the mussels' response to naturally occurring *M. aeruginosa*, which occurred as colonies in the larger size fraction. Measuring Chl *a* concentrations in the water column and on the entire beaker contents after resuspending all settled material at the end of the experiment allowed us to do a mass balance of mussel-induced changes to distinguish between what Chl *a* was filtered and what was assimilated. Typically, two 200-mL samples were taken by wide-bore volumetric pipette from the water column and from the mixed beaker contents for Chl *a* analyses.

Filtering (clearance) rate (F) for particle removal in each experimental container was calculated by (Vanderploeg et al. 1995)

$$(1) \qquad F = (V/nt)\ln(C_{wc}/Z_{wc})$$

where C_{wc} is the mean Chl *a* concentration in the water column at the end of the experiment in control containers, Z_{wc} is the Chl *a* concentration in the water column of an experimental container at the end of the experiment, V is the volume of water in the container during the experiment, n is the number of mussels, and t is the duration of the experiment.

We calculated this filtering rate for both chlorophyll size fractions and used the filtering rate on the preferred size fraction (F_{pref}), i.e., the size fraction having the highest filtering rate, to estimate pumping rate (Vanderploeg et al. 1995). The F_{pref} would closely approximate pumping rate if particles in the preferred size fraction were efficiently filtered and if feces and pseudofeces production added back to the water column in this size category was low (Vanderploeg et al. 1995).

The product of pumping rate and "average" concentration of Chl *a* in the water column ($\bar{C}_{wc}$) gave Chl *a* removed, i.e., the capture rate (CR)

$$(2) \qquad CR = F_{pref}\bar{C}_{wc}$$

$\bar{C}_{wc}$ was calculated with the formula given by Frost (1972):

$$(3) \qquad \bar{C}_{wc} = (Z_{wc} - C_0)/(\ln Z_{wc} - \ln C_0)$$

where C_0 is the mean initial concentration of chlorophyll in all containers.

To calculate assimilation rate and the corresponding clearance rate for assimilated material, we used the following approach. Using equations analogous to eqs. 1 and 3, a filtering rate (F_T) for total Chl *a* in the beakers (after mixing beaker) and "average" concentration of Chl *a* ($\bar{C}_T$) in each experimental beaker were calculated. Analogous to eq. 2, we calculated assimilation rate (A) as

$$(4) \qquad A = F_T\bar{C}_T.$$

Using A given by eq. 4 and the basic definition of clearance rate (eq. 2), the filtering rate for Chl *a* assimilated was calculated from the Chl *a* available in the water column by

$$(5) \qquad F_A = A/\bar{C}_{wc}.$$

All filtering rates were normalized to AFDW of the mussels used in the experiments as well as to gill surface area calculated from the gill area to mussel length relationship given by Lei et al. (1996). This latter normalization, which was used throughout this paper, was chosen to provide filtering estimates that were independent of mussel size (Kryger and Riisgård 1988; Lei et al. 1996) and condition (weight for a given length), which can change with

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site, reproductive status, and season (Nalepa et al. 1993; H.A. Vanderploeg, unpublished data).

To calculate A or CR as a percentage of mussel body carbon, we converted Chl *a* feeding rate to carbon feeding rate by multiplying Chl *a* feeding rate by the ratio of seston particulate carbon (POC) to Chl *a* concentration. AFDW of mussels was converted to carbon content by multiplying AFDW by 0.524 (Nalepa et al. 1993).

All feeding rate variables were calculated for each size fraction and for the total of both size fractions. The size fraction referred to was put in parentheses next to the variable, and when no reference to a size fraction is presented, it refers to the variable calculated from the sum of chlorophyll in both size fractions. Note that A, by definition, equals $A(<53) + A(>53)$, but F_A does not usually equal $F_A(<53) + F_A(>53)$. The relative F_A values of the two size fractions provide estimates of selectivities for the total filtering–ingestion–assimilation process for algae in the two size-classes (Vanderploeg 1994). Unless there is significant survival of algae during gut passage, relative F_A values closely approximate selectivities for ingestion. All feeding rate variables were tested to see if they were significantly different ($P < 0.05$) from zero using a two-tailed t test.

Design for experiments with *Microcystis* in natural seston

The goal of the experiments with natural seston that contained *M. aeruginosa* was to determine feeding rate variables on natural *M. aeruginosa* colonies in contrast with other seston. Two experiments were conducted using unmodified seston: Saginaw Bay (SB) seston (11 July 1995) and Lake Erie (LE) seston (21 September 1995) (Table 1). Other experiments added *Rhodomonas minuta* or *Cryptomonas ozolini*, small (5 µm and 8 µm) highly digestible cryptophytes of high food quality (Vanderploeg et al. 1996), to unmodified seston or to >53, 53–153 µm, or >153 µm size fractions of seston dominated by *M. aeruginosa* (Table 1). Experiments involving cryptophyte addition were designed to show filtration and rejection abilities of the mussels when offered a small desirable alga in the presence of naturally occurring *Microcystis*.

In the experiment "SB seston > 53 µm + *Rhodomonas*," we gently concentrated natural seston on a 53-µm screen and added it and *Rhodomonas* to 0.2-µm-filtered lake water to produce the chlorophyll size distribution in Table 1. In the experiment "LE seston + *Rhodomonas*," *Rhodomonas* was added to LE seston to enrich the <53 µm size fraction final concentration of ~4 µg Chl $a \cdot L^{-1}$. Both of these enrichment experiments were performed 1 day following experiments with the natural seston, and mussels were kept in natural seston before they were added, without acclimation, to the cryptophyte-amended suspensions.

The experiments performed with SB seston + *Cryptomonas* on 15 August 1997 were sequentially performed with the same mussels previously acclimated to natural seston for ~1 day (Table 1). The first experiment used seston concentrated on a 153-µm screen added to 0.2-µm-filtered lake water enriched with *Cryptomonas*. In the second experiment, we added seston in the size range 53–153 µm by collecting seston on a 53-µm screen that had first been filtered through a 153-µm screen to see if mussels would better ingest "smaller" than "larger" *Microcystis* colonies (Table 1). The mussels were acclimated for 1 h with the experimental mixture of seston and *Cryptomonas* prior to each experiment.

Design of experiments with laboratory cultures of *Microcystis*

Experiments with three different laboratory strains of *M. aeruginosa* were performed to evaluate effect of strain, microcystin concentration, and colony size on mussel feeding and behavior. We selected strains having different microcystin concentration (Table 2) that maintained some degree of colony formation in culture to allow examination of mussel feeding on large versus small colonies or individual cells. CCAP 1450/11 contained no microcystin (Table 2). We chose PCC 7820 because it is known to be toxic and severely depress feeding and survival in *Daphnia magna* (Nizan et al. 1986). LE-3, the most toxic strain in this study (Table 2), was isolated from the *M. aeruginosa* bloom on Lake Erie in September 1995 (Brittain et al. 2000). The work with the LE-3 strain and other strains was particularly important for examining feeding on *Microcystis* colonies in the <53 µm fraction because colonies in this size category were not available in natural seston.

Acclimation to these experimental mixtures of laboratory cultures was similar to the reacclimation procedure described for natural seston; however, acclimation periods varied according to experiment (Table 2). *Dreissena polymorpha* used in these experiments were maintained in the laboratory on Algae Diet C (preserved *Thalassiosira*, *Skelatonema*, *Isochrysis*, and *Chaetoceros*) (Coast Seafoods Co., South Bend, Wash.) supplemented with *C. ozolini* in large well-mixed and aerated aquaria (Vanderploeg et al. 1996).

Experiments 1 and 2 looked at feeding rate variables for a single strain of *M. aeruginosa* (Table 2). Experiment 1 with CCAP 1450/11, in addition to examining the effect of a particular strain type, examined the response of mussels to a high concentration of *Microcystis*, as found during a bloom (Table 2). Experiment 2, as well as Experiments 3 and 4, used much lower algal concentrations as might occur before a bloom to determine selectivity processes leading to bloom formation.

The multitreatment Experiments 3 and 4 each used the same set of mussels with three different algal treatments (Table 2). Experiment 3 evaluated the mussel response to different-sized colonies of the LE-3 strain of *M. aeruginosa*. Treatment 3A explored the mussels' response to the LE-3 strain presented as small colonies (<53 µm size fraction) produced by screening the suspension through a 53-µm screen. Treatment 3B examined the effects of a more even distribution of colony sizes. Treatment 3C with *Cryptomonas* was a control to show the effects of a highly desirable alga.

Experiment 4 examined the response of mussels to mixture of small colonies of the LE-3 strain of *M. aeruginosa* and *Cryptomonas*. Treatment 4A with *Cryptomonas* was used to establish a baseline of effects of high-quality food alone. Experiment 4B was done to establish what the effects of small colonies (mean cells per colony = 4.3) of *M. aeruginosa* strain LE-3 offered alone. These colonies were prepared by pouring the culture through a 37-µm screen. Treatment 4C examined the effects of 50:50 mixture (based on cell volume concentration measured with a Coulter counter (Vanderploeg et al. 1995)) of small colonies of LE-3 *M. aeruginosa* and *Cryptomonas* on mussel feeding.

Results

Chlorophyll, phytoplankton composition, and microcystin in Saginaw Bay

Zebra mussels started significantly recruiting to Saginaw Bay during the late summer and fall of 1991 (Nalepa et al. 1995). Monitoring results for 1992–1996 revealed that in every year except 1993, there was a dramatic clear-water phase in spring followed by relatively high Chl *a* concentration during the summer (Fig. 1). In 1993, the clear-water phase lasted throughout the summer.

In 1990, before the zebra mussels invaded the bay, the cyanobacterium *Oscillatoria* comprised 35–40% of phytoplankton biomass during May and September, but it or other cyanobacteria were not found in October. In 1991, cyanobacteria were rare (0–10% of the biomass) and *Microcystis* was not found. In contrast, *M. aeruginosa* was an important

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Table 1. Experimental conditions for zebra mussels feeding on naturally occurring *Microcystis* in seston from Saginaw Bay (SB) and Lake Erie (LE) on mixtures with *R. minuta* and *C. ozolini*.

Experiment	Date	Size fraction (μm)	$\bar{C}_{wc}$ (μg·L^{-1})	*Microcystis* biomass (%)	Microcystin concentration in *Microcystis* (μg·μg Chl a^{-1})	*Microcystis* colony size (cells·colony^{-1})	
						Mean	Range
SB seston	11 July 1995	<53	4.4				
		>53	19.3	82			
		Total	23.7	67	0.25	2 130	300 – 10 000
SB seston > 53 μm + *Rhodmonas*	12 July 1995	<53	6.1				
		>53	17.1	82	0.25	2 130	300 – 10 000
		Total	23.2				
LE seston	21 Sept. 1995	<53	1.3				
		>53	36.6				
		Total	37.9	76		3 230	800 – 10 000
LE seston + *Rhodmonas*	22 Sept. 1995	<53	3.5				
		>53	16.6				
		Total	20.1	76		3 230	800 – 10 000
SB seston > 153 μm + *Cryptomonas*	15 Aug. 1997	<53	2.8				
		>53	1.9	99	0.30	1 320	200 – 4 500
		Total	4.9				
SB seston 53–153 μm + *Cryptomonas*	15 Aug. 1997	<53	3.2				
		>53	1.7	82	0.30	207	20 – 700
		Total	4.9				

Note: $\bar{C}_{wc}$ is the average Chl *a* concentration available to the mussels during the experiment; SE/$\bar{X}$ was in all cases <5%. All experiments were run at 20–21°C.

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Table 2. Experimental conditions for zebra mussels grazing on different *M. aeruginosa* strains and *C. ozolini* offered alone or together.

Strain or mixture and comments	Experiment	Acclimation period (h)	Temperature (°C)	Microcystin concentration (μg·μg Chl a^{-1})	Size fraction (μm)	$\bar{C}_{wc}$ (μg·L^{-1})
CCAP 1450/11[a]	1	4	25	0	<53	10.8
					>53	1.0
					Total	11.8
PCC 7820[b]	2	70	17	0.22	<53	3.9
					>53	0.5
					Total	4.4
LE-3[c] (<53 μm)	3A	18	20	0.66	Total[e]	5.6
LE-3[c]	3B	2	20	0.66	<53	0.8
					>53	0.7
					Total	1.5
Cryptomonas	3C	1	20	0	Total[e]	7.8
Cryptomonas	4A	18	20	0	Total[e]	3.0
LE-3[c] (<53 μm)	4B	18	20	0.66	Total[e]	3.8
50:50 mixture of LE-3 (<53 μm) and *Cryptomonas*	4C	19	20	0.66[d]	Total[e]	4.2

Note: Experiments with same number but different letter designations were run with the same mussels on the same or consecutive days in the indicated order. LE-3 (<53) refers to the LE-3 strain that was poured through a screen to produce colonies <53 μm; $\bar{C}_{wc}$ is the average Chl *a* concentration available to the mussels during the experiment; SE/$\bar{X}$ was in all cases <5%.

[a]Culture Collection of Algae and Protozoa, Ambleside, U.K.

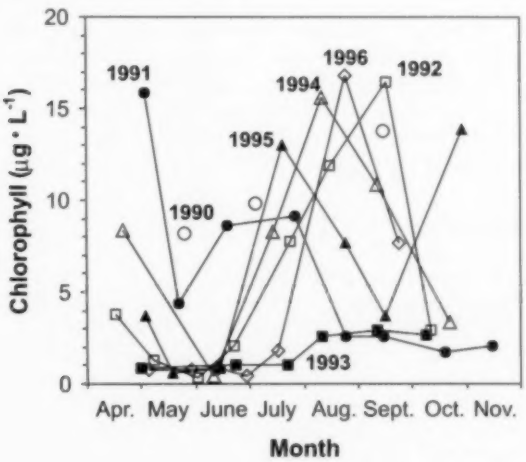
[b]Pasteur Culture Collection, Paris, France.

[c]Lake Erie isolate of Wayne Carmichael (Brittain et al. 2000).

[d]Microcystin concentration in *Microcystis* fraction.

[e]*Cryptomonas* or LE-3 *Microcystis* fell within the <53 μm size fraction.

Fig. 1. Seasonal chlorophyll concentrations at Station 5 in Saginaw Bay during years before and after zebra mussels invaded Saginaw Bay. Data are means that have a precision (SE/$\bar{X}$) of better than 5%.



component (>40% of biomass on at least one date) of the algal community during 1992, 1994, and 1995 and tended to dominate during summer and early fall (Fig. 2). During blooms in Saginaw Bay and Lake Erie, the colonies were quite large, having 300 – 10 000 cells·colony^{-1} (Table 1). During nonbloom periods, colonies were smaller (Tables 1 and 3); however, in all cases, *M. aeruginosa* was primarily found in the >53 μm size fraction. At all times sampled (Tables 1 and 3), *M. aeruginosa* was toxic, and during experiments, microcystin concentration was about the same (Table 1). Although the microcystin concentration in the Lake Erie bloom was not measured, the isolate from the bloom had a high concentration of microcystin (Table 2). In September 1992, other gelatinous colony-forming cyanophytes, *Agmenellum* and *Synechococcus*, combined with *Microcystis* to account for 80% of the algal community.

Experiments with natural seston

The mussels in all experiments except the SB experiment (with unmodified seston) exhibited high values of F(<53) that were significantly different from zero (Fig. 3*a*). A similar pattern was seen for F_A(<53), except that F_A(<53) was appreciably lower than $F_{(<53)}$ in the LE experiment. In contrast, F(>53) was low and F_A (>53) was very low (Fig. 3) in all experiments. Only in the case of the experiment "SB seston 53–153 μm + *Cryptomonas*" were both F(>53) and F_A(>53) significantly different from zero. Since *M. aeruginosa* dominated the >53 μm fraction (Table 1), this implied that the response to the >53 μm fraction represented the response to *Microcystis*. That F(>53) was much less than F(<53) implied that an appreciable portion of CR not assimilated was returned to the water column under the mixing regime of this experiment. The low or zero values of F_A(>53) (relative to high values of F_A(<53)) imply very low or zero selectivity for the *M. aeruginosa* colonies.

The high values of F(<53) (= pumping rate) and the very high concentrations of Chl *a* in the >53 μm fraction in the "SB seston > 53 μm + *Rhodomonas*," "LE seston," and "LE seston + *Rhodomonas*" experiments (Table 1) led to very high CRs (CR = 200–380% C·day–1, Fig. 3*c*), most of which was not assimilated (Fig. 3*d*) but, as shown by video observations, was expelled in the form of pseudofeces primarily composed

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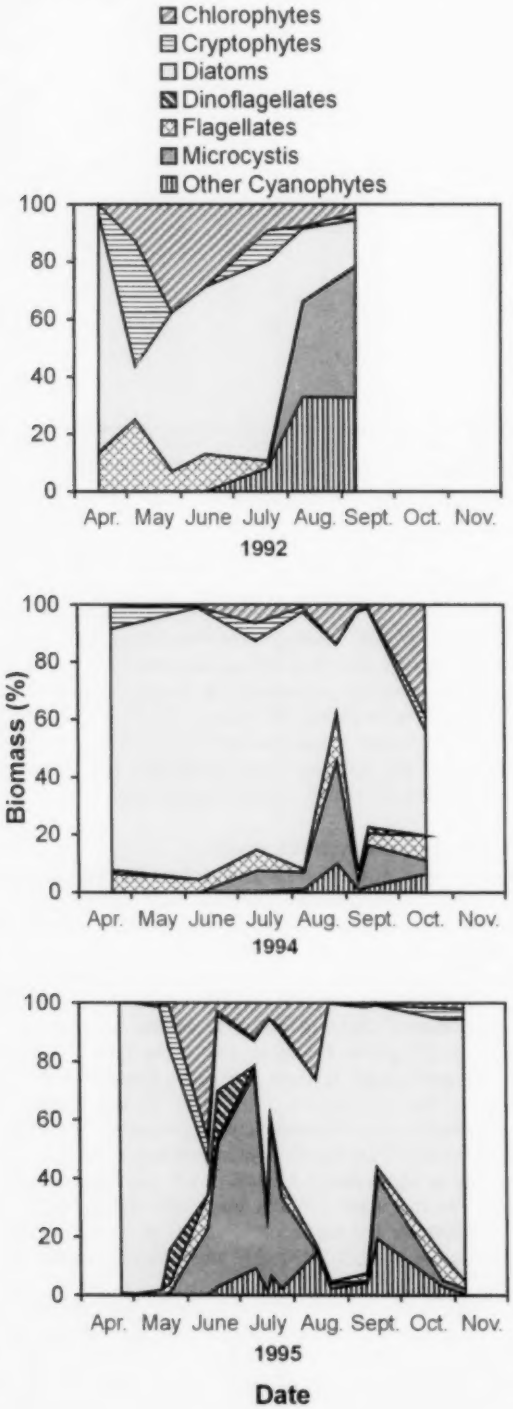
Fig. 2. Seasonal phytoplankton composition expressed as relative carbon biomass at Station 5 in Saginaw Bay during years of *Microcystis* dominance.



of *Microcystis* colonies (H.A. Vanderploeg, unpublished data). These pseudofeces were very unconsolidated, a mass of loosely aggregated colonies that tended to break down into their constituent colonies as they were expelled from the incurrent siphon (H.A. Vanderploeg, unpublished data).

Large and significant values of A were in all cases due to the large contribution of $A(<53)$. Because A is the product of filtering rate and Chl *a* concentration (eq. 5), the large but not significant values of $A(>53)$ and A for the LE experiment were due to the product of low but variable $F_A(>53)$ values (Fig. 3*b*) and very large Chl *a* concentrations in the >53 μm size fraction (Table 1).

Experiments with laboratory cultures

Mussels exhibited high values of F, F_A, and A when feeding on the toxic (Table 2) PCC 7820 strain of *M. aeruginosa* (Experiment 2, Fig. 4). The $F(<53)$, $F(>53)$, $F_A(<53)$, $F_A(>53)$, and A of PCC 7820 were all equal to or greater than the respective values of for *Cryptomonas* in the other experiments (Fig. 4). Thus, the mussels treated this toxic strain as a preferred food, and selectivity (F_A) for large colonies (>53 μm) was nearly as high as for small colonies (<53 μm).

The F and F_A of the nontoxic CCAP 1450/11 strain (Table 2) were considerably lower than the respective values for PCC 7820 (Fig. 4). Both $F(>53)$ and $F_A(>53)$ of the CCAP 1450/11 strain were negative, and $F(<53)$ and $F_A(<53)$ were considerably lower than the respective values for PCC 7820 or *Cryptomonas*; however, A was about the same. Much of the material filtered was ingested because both CR and A had similar values. In Experiment 3, F, F_A, and A were low or not significantly different from zero for the LE-3 strain of *Microcystis*, whether presented as colonies <53 μm (Treatment 3A) or distributed between size-classes (Treatment 3B) (Fig. 4; Table 2). In contrast, after only 1 h of acclimation, F, F_A, and A were high for *Cryptomonas* (Treatment 3C, Fig. 4). In Experiment 4, F, F_A, and A were high for *Cryptomonas*, not significantly different from zero for the LE-3 strain of *Microcystis*, and low for the mixture of the LE-3 strain of *Microcystis* plus *Cryptomonas* (Fig. 4).

Discussion

Strain- and size-specific response

Mussel response to *M. aeruginosa* was strain specific. The toxic PCC 7820 and nontoxic CCAP 1450/11 strains were readily filtered (F_A) or assimilated (A) at rates comparable with those of *Cryptomonas*, a highly desirable food. It seems probable that low $F(<53)$ and $F_A(<53)$ values for the CCAP 1450/11 strain of *M. aeruginosa* resulted from the Chl *a* concentration in this experiment being above the incipient limiting concentration (ILC). Sprung and Rose (1988) found that the ILC for zebra mussels feeding on *Chlamydomonas* expressed in algal volume units was ~2 $mm^3 \cdot L^{-1}$; average concentration of CCAP 1450/11 was equivalent to 7.2 $mm^3 \cdot L^{-1}$. It is also possible that there was some inhibition of filtering rate due to this culture being in early stationary phase rather than exponential phase as were all other cultures. The negative values of $F(>53)$ and $F_A(>53)$ probably do not indicate a lack of ingestion of colonies in the >53 μm fraction. Instead, this is probably a typical case of the particle-production problem that arises when high food

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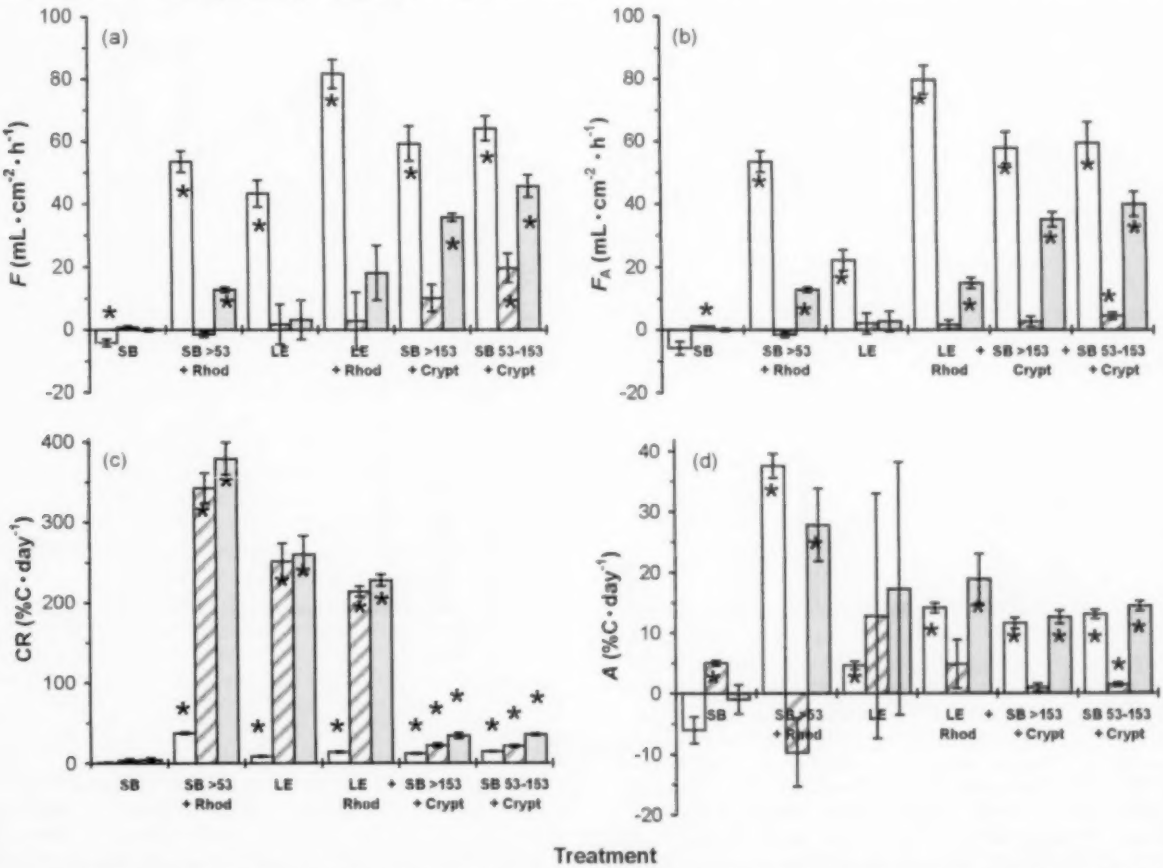
Table 3. Colony size and microcystin concentration of *M. aeruginosa* at Station 5 in Saginaw Bay.

	Microcystis colony size (cells·colony^{-1})		Microcystin concentration	
Date	Mean	Range	Water (μg·L^{-1})	*Microcystis* (μg·μg Chl a^{-1})
20 June 1995	691	100–1000	0.10	0.14
11 July 1995	2130	300 – 10 000	3.5	0.25
15 Aug. 1995	71	50–100	0.10	0.20

Note: Values for water are totals for the particulate phase.

Fig. 3. Effect of naturally occurring *M. aeruginosa* on (*a*) filtering rates (*F*), (*b*) filtering rates for assimilated chlorophyll (F_A), (*c*) capture rates (CR), and (*d*) assimilation rates (*A*) in experiments (Table 1) with seston from Saginaw Bay (SB), Lake Erie (LE), and mixtures of seston and laboratory cultures of *Rhodomonas* (Rhod) and *Cryptomonas* (Crypt). In all cases, *M. aeruginosa* dominated the >53 μm size fraction. For each experiment, results are shown from left to right for <53 μm (open bars), >53 μm (hatched bars), and the total (shaded bars) size fractions. Means and standard errors for the four replicate experimental beakers are shown, and asterisks indicate values significantly different ($P < 0.05$) from zero.



concentration in one size category is transformed into particles in another size category having few particles to begin with (Vanderploeg et al. 1984). Here, concentration of Chl *a* in the >53 μm fraction was very low, and high pseudofeces production resulting from high CR(<53) added much Chl *a* to the >53 μm fraction.

The F_A values for *Cryptomonas* and PCC 7820 (90–100 mL·cm^{-2}·h^{-1}) correspond closely to the high filtering rate (84–114 mL·cm^{-2}·h^{-1}) reported by Kryger and Riisgård (1988) for *Chlorella* at 20°C under "ideal" laboratory conditions. Assimilation rates of 30–40% C·day^{-1} observed in our experiments for *Cryptomonas* and the PCC 7820 and CCAP 1450/11 strains of *M. aeruginosa* were considerably higher than the maximum of 4.5% C·day^{-1} reported by Walz (1978) for the pennate diatom *Nitzschia*. The experiment with the PCC 7820 strain showed that both large and small size fractions of *M. aeruginosa* were ingested (and assimilated).

In contrast, F_A and *A* of the small colonies of the LE-3 strain and large colonies of *M. aeruginosa* of Saginaw Bay or Lake Erie seston were low or zero. Since the LE-3 strain was isolated from the Lake Erie bloom, we can argue that Lake Erie *M. aeruginosa* of all sizes were unpalatable to the mussels. The F_A and *A* of *M. aeruginosa* from Saginaw Bay were low, whether presented as that found in natural seston

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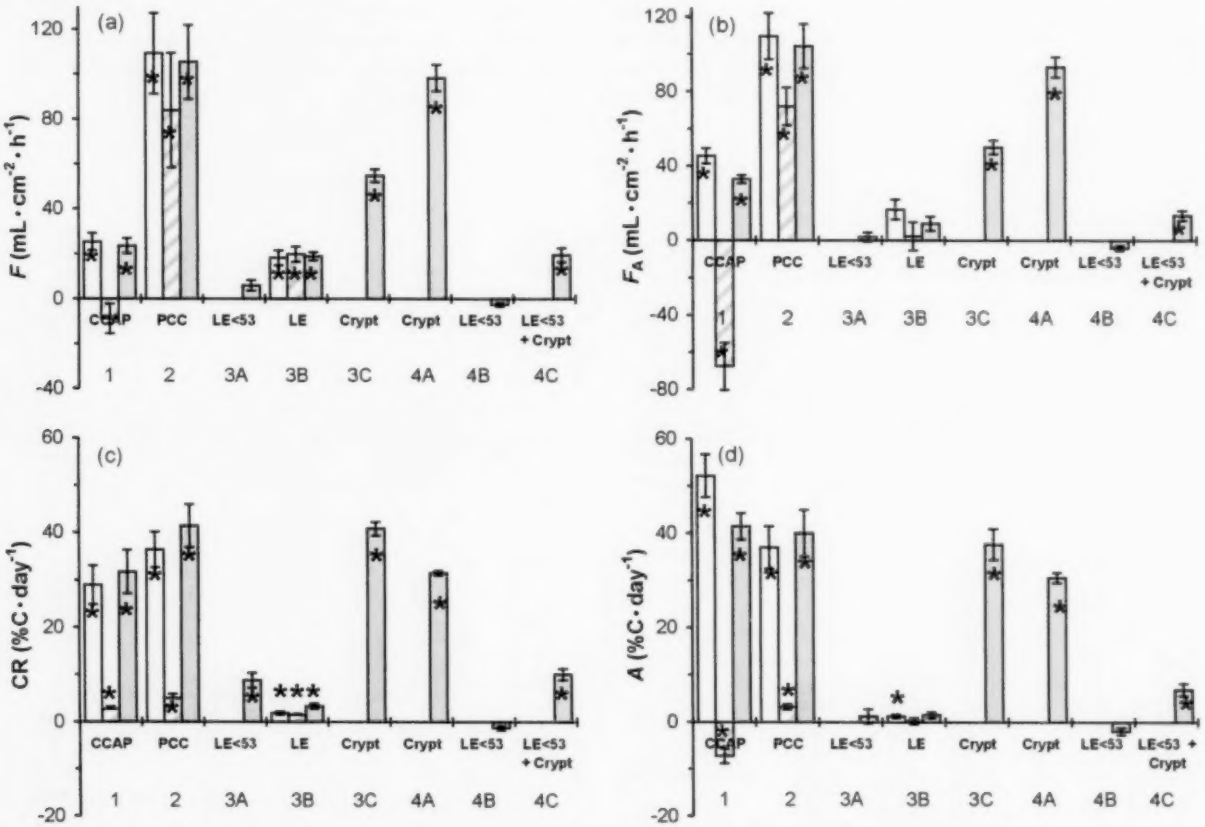
Fig. 4. (*a*) Filtering rates (*F*), (*b*) filtering rates for assimilated chlorophyll (F_A), (*c*) capture rates (CR), and (*d*) assimilation rates (*A*) in four experiments (Table 2) with individual strains of *M. aeruginosa* in comparison with *C. ozolini* (Crypt) and in mixture to evaluate the effects of *M. aeruginosa* strain type and size. *Microcystis aeruginosa* strain types: CCAP, Culture Collection of Algae and Protozoa (Ambleside, U.K.) strain 1450/11; PCC, Pasteur Culture Collection (Paris, France) strain 7820; LE, LE-3 strain. The experiment numbers referred to in Table 2 are shown on the abscissa, and the uppercase letters refer to treatments within an experimental series with the same mussels. Means and standard errors for the four replicate experimental beakers are shown, and asterisks indicate values significantly different ($P < 0.05$) from zero. In experiments labeled CCAP (Experiment 1), PCC (Experiment 2), and LE (Treatment 3B), a broad size range of colonies (Table 2) was presented and results are given for <53 μm (open bars), >53 μm (hatched bars), and the total (shaded bars) size fractions. In experiments with small-sized colonies of LE-3 *M. aeruginosa* (labeled LE <53), *C. ozolini* (Crypt), or mixtures of Crypt and LE <53, all cells or colonies appeared in the <53 μm fraction and are presented as "total" results (shaded bars).



or that in the restricted size ranges of 53–153 and >153 μm. Experiments with other large colony-forming algal species and microzooplankton indicated that colonies in the size range of 53–153 μm, although not filtered at maximal rates, would not present a great impediment to feeding (e.g., MacIsaac et al. 1991; Horgan and Mills 1997; Bastviken et al. 1998). Ten Winkel and Davids (1982) noted that algae as large as 750 μm could be ingested. Thus, although it is possible that very large colonies may be impossible for *Dreissena* to ingest, chemical quality or toxicity was important to the mussels' response. Poor chemical quality was suggested as the reason for the much lower selectivity for 25-μm-diameter colonies of the cyanobacterium *Chroococcus* relative to algae of the same size (Ten Winkel and Davids 1982).

Experiments of Lavrentyev et al. (1995), Bastviken et al. (1998), and Baker et al. (1998) are consistent with our conclusions of the importance of *M. aeruginosa* strain type on mussel response. Lavrentyev et al. (1995) observed zero net clearance rates on *Microcystis* in screened (40 and 53 μm) and unscreened Saginaw Bay seston in September and October 1994, when 40% of the algal biomass was *Microcystis*. Bastviken et al. (1998) showed that "net" clearance rate ($\sim F_A$) of natural colonial *Microcystis* (with greatest linear dimension of 49 μm) in Hudson River seston was about 25% of that of the preferred foods, which were ambient cryptophytes and unicellular cultured *M. aeruginosa* (Carolina Biological Supply) added to the seston.

In contrast, Baker et al. (1998) reported that unicells of the nontoxic UTEX 2386 and toxic UTEX 2385 strains of *M. aeruginosa* were cleared at a rate greater than green algae and diatoms. Clearance rates calculated for their 20-mm mussels for the nontoxic strain were ~45 ± 2 mL·cm^{-2}·h^{-1} at

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16°C, which falls between the values reported for PCC 7820 and CCAP 1450/11. Respective concentrations of algae expressed on a volumetric basis were 3.4, 2.3, and 7.2 $mm^3 \cdot L^{-1}$, where the first value refers to initial concentration and the other two are average concentrations from Table 2. Based on the expected lowered filtering rate due to an ILC of ~2 $mm^3 \cdot L^{-1}$ (Sprung and Rose 1988), these results represent feeding rates on desirable foods.

The low clearance rates that Baker et al. (1998) observed on green algae and diatoms were probably a result of their being above the ILC; the diatoms and green algae were much larger than *Microcystis* and all algae were presented at a high initial concentration (10^5 cells·mL^{-1}). In the case of the diatom *Cyclotella*, the initial concentration of 10^5 cells·mL^{-1} corresponds to 52 $mm^3 \cdot L^{-1}$.

Role of microcystin

Microcystin concentrations in *M. aeruginosa* from Saginaw Bay were midrange (0.14–0.30 μg·μg Chl a^{-1}) of values reported for *M. aeruginosa* in German Lakes (Sivonen and Jones 1999). The 11 July 1995 microcystin concentration of 3.5 $\mu g \cdot L^{-1}$ in Saginaw Bay was the highest concentration found in the particulate phase in our study of Saginaw Bay. Much higher concentrations (10–100 $\mu g \cdot L^{-1}$) have been found in more eutrophic systems with higher *Microcystis* concentrations (Sivonen and Jones 1999). If the microcystin content of the LE-3 strain were representative of *M. aeruginosa* in Lake Erie from which it was isolated, the toxin concentration found in Lake Erie *Microcystis* in September 1995 would have been estimated at 24 $\mu g \cdot L^{-1}$ and would have fallen within the high end of the range reported for lakes (Sivonen and Jones 1999).

The high values of F_A and A for PCC 7820 were surprising given its well known tendency to "block" ingestion in *Daphnia* and cause rapid mortality from the small amount ingested (Nizan et al. 1986). PCC 7820 is also known to be toxic in mouse bioassays, and its extracts are toxic to copepods, which normally reject it and thus avoid ingestion (DeMott and Moxter 1991). The toxic strain used by Baker et al. (1998) was less potent than PCC 7820 in that ingestion was not as much suppressed in *Daphnia* and survival was high (Nizan et al. 1986). It would be tempting to explain *Dreissena*'s low F_A and A on the LE-3 isolate from Lake Erie to be a result of its high microcystin concentrations relative to other strains. However, it has been argued that toxicity of *Microcystis* to zooplankton may not be actually caused by microcystin but another secondary compound termed *Daphnia*-toxic compound (DTC) (Jungmann and Benndorf 1994). In addition, the rejected *M. aeruginosa* from Saginaw Bay had a microcystin concentration about the same as that for PCC 7820.

Perhaps the low values of $F(<53)$ and $F_A(<53)$ of the mussels in Saginaw Bay seston represented a chronic effect of a long-term exposure to a high concentration of toxic *Microcystis*. Toxic *Microcystis* had dominated the algal community at Station 5 in Saginaw Bay for 3 weeks before this experiment. Video observations showed that in this experiment, the mussels were actively filtering only 54% if the time whereas in all other experiments, including the one with Saginaw Bay seston enriched with *Rhodomonas*, were filtering 93–97% of the time (H.A. Vanderploeg, unpublished data).

Sorting abilities of mussels

The depression of feeding rate seen in the mixture of LE-3 *Microcystis* and *Cryptomonas* (Experiment 4) probably indicates an inability to sort out small *Microcystis* colonies from *Cryptomonas*. The depression in filtering rate was also seen in mussel feeding activity (H.A. Vanderploeg, unpublished observations). Mussels exposed to *Cryptomonas* alone were actively filtering 92% of the time whereas they were filtering only 41–52% of the time when given small LE-3 *Microcystis* colonies or the LE-3 *Microcystis* + *Cryptomonas* mixture, and few pseudofeces were produced (H.A. Vanderploeg, unpublished data).

Mussels do have the ability to sort out different particles and even different algal species. Ward et al. (1998) showed that the marine mussel *Mytilus* can sort out detritus of *Spartina* (3–20 μm) from similarly sized *Rhodomonas* (6–13 μm) and enrich the pseudofeces with a higher concentration of *Spartina* relative to *Rhodomonas*. Baker et al. (1998) showed that when various high concentration mixtures of large green algae and diatoms were presented to mussels with small unicells of nontoxic *M. aeruginosa*, the pseudofeces were enriched with the large algae relative to *Microcystis*. Furthermore, as noted above, zebra mussels had a lower selectivity for 25-μm-diameter *Chroococcus* relative to other algae of the same size (Ten Winkel and Davids 1982). However, no one has shown that small (4–6 μm) algal species of nearly the same size can be sorted. The depression in feeding rate in mussels was similar to that in filter-feeding cladocerans when *Microcystis* was paired with similar-sized desirable green algae (Lampert 1982; Fulton and Paerl 1987). It is unknown how much of the small colonies of *Microcystis*, relative to other food, is required to cause a significant drop in mussel feeding.

Ecosystem implications

Did zebra mussel grazing promote toxic Microcystis *dominance?*

We do not have extensive series of algal community structure and toxin concentration for either Saginaw Bay or Lake Erie that cover a long period before and after the zebra mussel invasions; however, some information is available. In 1990 and 1991, before zebra mussel dominance in Saginaw Bay, *Microcystis* was not abundant. *Microcystis aeruginosa* dominated the algal community of Saginaw Bay during 1992, during brief periods during 1994, and for much of the summer during 1995. In all times sampled in 1995, *M. aeruginosa* contained microcystin. *Synechococcus* was also present in September 1992 and it, in addition to *M. aeruginosa*, could have been toxic because it is a known microcystin producer (Domingos et al. 1999). Furthermore, it is possible that our estimates of *Microcystis* abundance may be underestimated. Lavrentyev et al. (1995) reported that *Microcystis* dominated 40% of the algal community in September and October 1994, and our 30-cm-diameter 64-μm-mesh plankton nets were clogged with large *Microcystis* colonies that may not have been adequately sampled by the 2–50 mL subsampled for making phytoplankton slides. Thus, *Microcystis* was not a dominant part of the phyto-

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Table 4. *Dreissena polymorpha* biomass (Nalepa et al. 1999; T.F. Nalepa, unpublished data) and grazing impact on desirable algae expressed as the fraction of the water column cleared (= mortality rate coefficient) in the inner portion of Saginaw Bay assuming a clearance rate of 10–30 mL·mg^{-1}·h^{-1} (≈38–129 mL·cm^{-2}·day^{-1} for mussels in our study), which were typical low and high values measured there by Fanslow et al. (1995).

	Year				
Variable	1992	1993	1994	1995	1996
Biomass (g·m^{-2})	43.33	3.15	2.17	2.87	9.38
Fraction of water column cleared (day^{-1})	2.04–6.12	0.15–0.45	0.10–0.30	0.14–0.42	0.44–1.32

plankton community 2 years before the invasion and was an important part three out five summers following the zebra mussel invasion.

In Lake Erie, cyanobacteria were a minor component of the phytoplankton before (1985–1987) and during the early expansion of the zebra mussel population (1988–1990) (Nicholls and Hopkins 1993). No *Microcystis* bloom of the magnitude of that occurring during September 1995 had occurred in the few years before or after this bloom. Such a bloom would have been obvious from shore or satellite imagery. These data from both Saginaw Bay and Lake Erie are consistent with mussels increasing the probability of a bloom, but *Microcystis* blooms are not a certainty.

Whether or not zebra mussels were responsible for *Microcystis* bloom promotion, they were not capable of controlling *Microcystis* concentrations. Marine mussels have been described as ecosystem stabilizers and eutrophication controllers (Herman and Scholten 1990) through their control of phytoplankton concentration by grazing. This control of phytoplankton concentration and resultant water clarity is often mentioned as ecosystem impacts of zebra mussels (e.g., Fahnenstiel et al. 1995; Caraco et al. 1997; Smith et al. 1998). *Microcystis* dominance in Saginaw Bay and Lake Erie and high summer chlorophyll concentrations in Saginaw Bay serve as a cautionary tale for the ideas of ecosystem stabilization or eutrophication control.

We estimated the potential collective impact of zebra mussels on mortality rate of desirable algae by calculating the fraction of water cleared (= mortality rate coefficient) by the mussels per day in the inner bay region of Saginaw Bay using biomass of mussels and weight-specific filtering rates given by Fanslow et al. (1995). Using a range of realistic upper and lower values of weight-specific filtering rates from Fanslow et al. (1995) resulted in mortality rate coefficients ranging between 0.10 and 6.12·day^{-1} over the study period (Table 4). Typical specific growth rates of phytoplankton in natural systems range between 0.2 and 0.5·day^{-1} in nutrient-limited systems with a maximum between 1 and 2·day^{-1} where nutrients are not limiting (Reynolds 1997). Measured specific growth rates in Saginaw Bay were 0.20–0.25·day^{-1} (Fahnenstiel et al. 1995). Since most of the mortality rates were in the range of specific growth rates or greater, mussel grazing could have been an important force in promoting *Microcystis* and other grazing-resistant cyanophytes and algae. In Saginaw Bay, mussels had the strongest hypothetical grazing impact on mortality during 1992, which was the first year the mussels were there in all seasons and the biomass was high. In 1994 and 1995, values were more modest but were about the same as algal growth rate. However, in the nonbloom years of 1993 and 1996, calculated grazing impact was also appreciable. The potential impact of mussel grazing was also high in western Lake Erie, where Bunt et al. (1993) estimated that mussel clearance of the water column was 0.4–1.0·day^{-1}, and a more recent estimate (H.A. Vanderploeg, unpublished data) puts it at ~1·day^{-1}.

High filtering rates, which would favor the selection process for *Microcystis*, were dependent on composition of the algal community. Experiments with natural *Microcystis* showed that $F(<53)$ and $F_A(<53)$ were greatest when cryptophytes were added to the seston. Particularly noteworthy is the zero or negative values of $F(<53)$ and $F_A(<53)$ in the experiment with Saginaw Bay seston that became very high with the addition of *Rhodomonas*. If selective rejection was operating as a mechanism for dominance of *Microcystis* in July 1995 in Saginaw Bay, then filtering rates had to be higher at an earlier time. The relatively high $F(<53)$ and $F_A(<53)$ for unmodified Lake Erie seston of 1995 probably follow because cryptophytes and various flagellates were the dominant component of the phytoplankton after *Microcystis*. Thus, composition of the algal community could have been a factor in whether a bloom occurred.

The production of loosely consolidated pseudofeces is consistent with the return of viable *Microcystis* to the water column for continued growth. Baker et al. (1998) showed that pseudofeces produced in various combinations of laboratory algal cultures, clay suspension, and laboratory-produced detritus were diffuse. Moreover, they showed that the algae in the pseudofeces were alive and continued to grow when cultured.

Did zebra mussels select for a toxic grazing-resistant M. aeruginosa *strain?*

That zebra mussels showed low filtering rates and negative behavioral responses to the LE-3 toxic strain but not to the PCC and UTEX toxic strains raises the possibility that there may have been a selection for a particularly toxic or unpalatable strain of *M. aeruginosa* in Lake Erie. This argument would also apply to Saginaw Bay, where *M. aeruginosa* was also toxic and unpalatable as measured by microcystin concentration and mussel response. It can be argued that this response is not just a question of microcystin content because the mussels would have ingested great quantities of microcystin in the experiments with the PCC 7820 strain. There was probably selection for large *M. aeruginosa* colonies because selective rejection was only efficient with large colonies.

Why were there no Microcystis *blooms in the Hudson River and shallow Dutch lakes?*

It is important to consider that other factors such as light intensity (Mur et al. 1999), nutrient ratios (e.g., Smith 1983), and water temperature (Robarts and Zohary 1987) can affect

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competitive interactions between *Microcystis* and other algae. It is not surprising that zebra mussel selective rejection did not lead to blooms of toxic nitrogen-fixing cyanobacteria such as *Aphanizomenon* and *Anabaena*, which would be favored under very low nitrogen to phosphorus ratios, because Lake Erie and Saginaw Bay have moderate and high ratios, respectively (Holland et al. 1995; Johengen et al. 1995). Blooms of large colonial *Aphanizomenon* have occurred in eutrophic (i.e., low nitrogen to phosphorus ratio) Oneida Lake in late summer and autumn since the establishment of the zebra mussel (Horgan and Mills 1997). *Microcystis* and many other cyanobacteria have growth optima above 25°C, whereas green algae and diatoms have optima considerably below 25°C (Robarts and Zohary 1987). Thus, *Microcystis* blooms will occur only in summer and be favored by warmer temperatures.

It would seem puzzling at first that *Microcystis* has not dominated in certain other systems such as the Hudson River (Caraco et al. 1997; Smith et al. 1998) or shallow Dutch lakes (Reeders et al. 1989; Reeders and Bij de Vaate 1990) if zebra mussel grazing was the cause of blooms in other locations. The disappearance, rather than promotion, of *Microcystis* in the Hudson River (Smith et al. 1998) may be related to strain type. The maximum fraction of the water column cleared by the mussels was estimated to be ~0.7·day^{-1} (Caraco et al. 1997; Smith et al. 1998), which is an appreciable rate for affecting phytoplankton composition through selective rejection. As noted above, Bastviken et al. (1998) showed that *Microcystis* colonies from the Hudson River were grazed at rates that were 25% of the maximum rate for preferred algae. Thus, grazing may not have been a force to favor *Microcystis*, especially in view of its slower growth rate (Reynolds 1997). We cannot be sure of the relative roles of toxicity or size in this lack of rejection, since the colonies in the Hudson River were relatively small (47 μm) and toxin concentration was not measured.

Was there less of a selective force for the development of large unpalatable *Microcystis* colonies in the Hudson River? Because *Microcystis* and other cyanobacteria are favored over green algae and diatoms at low light intensities, light intensity rather than zooplankton grazing, which shapes summer phytoplankton community structure in nondreissenid lakes (Sommer et al. 1986), may have been the primary selective force in this turbid freshwater estuary. Light intensity was relatively unaffected in Hudson River because suspended sediments, although removed and packaged up in feces and pseudofeces by the mussels, were resuspended in this high-energy environment (Caraco et al. 1997). If light intensity and not grazing was the selective force, then *Microcystis* in this system may not have been preadapted for grazing resistance.

The lack of *Microcystis* blooms in Dutch Lakes IJsselmeer and Markermeer following zebra mussel introduction simply may have been a result of mussels not having a great enough grazing impact in these eutrophic lakes. The fractions of water cleared by the mussels in Lakes IJsselmeer and Markermeer were 0.09 and 0.06·day^{-1}, respectively.

In addition to selection for grazing-resistant algae or cyanobacteria, mussel filtering might promote selection of rapidly growing species that can grow faster than the mortality imposed by mussel clearance of the water column. This strategy would be most effective where nutrient concentrations are sufficient to promote rapid algal growth. Perhaps this scenario sometimes occurs in western Lake Erie, where phosphorus concentration is higher than in Saginaw Bay (Holland et al. 1995; Johengen et al. 1995). We do not know enough about the relationship between nutrient concentration and standing stock of zebra mussels to predict grazing pressure in different systems to evaluate grazing resistance versus growth rate strategies. Possibly, the lower incidence of blooms in Lake Erie compared with Saginaw Bay results from success of the rapid-growth strategy in most years.

We have shown that *Microcystis* is selectively rejected by zebra mussels and that selective rejection can be a large selective force for *Microcystis* or other grazing-resistant algae and cyanobacteria in shallow systems where zebra mussel density is high. Because the selective rejection mechanism requires that *Microcystis* be returned to the water column, the mechanism would work best in turbulent systems such as large shallow bays and lakes.

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Paleoenvironmental inference models from sediment diatom assemblages in Baffin Island lakes (Nunavut, Canada) and reconstruction of summer water temperature

Ernest H. Joynt III and Alexander P. Wolfe

Abstract: Diatoms from the surface sediments of 61 lakes on Baffin Island, Nunavut, were identified, enumerated, and interpreted quantitatively. The samples span a latitudinal transect from 62 to 74°N, reflecting climatic and vegetational gradients that range from low Arctic to transitional mid-Arctic to high Arctic. While the sampled lakes encompass both predominantly maritime and continental climatic regimes, sites have been deliberately restricted to basins in Precambrian crystalline terrain to mitigate the edaphic consequences of alkaline sedimentary bedrock. Canonical correspondence analysis, using forward selection and Monte Carlo permutation tests, identified pH, conductivity, summer lake water temperature, and mean annual air temperature as significant environmental controls over diatom assemblages. Using weighted-averaging regression and calibration, predictive models for these parameters have been developed. When applied to down-core assemblages, the summer lake water temperature model provides realistic reconstructions when compared with other paleoenvironmental records. Over the past 5000 years, the amplitude of reconstructed summer lake water temperature is on the order of 4.0°C, expressed primarily as progressive Neoglacial cooling culminating in the Little Ice Age. Diatom-inferred summer water temperatures have increased by 2.0°C in the past 150 years, again in agreement with independent paleoclimatic reconstructions.

Résumé : Nous avons identifié, énuméré et interprété quantitativement des échantillons de diatomées prélevés dans les sédiments superficiels de 61 lacs de l'île de Baffin, au Nunavut. Les prélèvements proviennent d'une zone s'étendant de 62 à 74°N et représentant des gradients de climat et de végétation, allant du bas Arctique à la transition du moyen Arctique au haut Arctique. Les lacs étudiés se situent dans des régions à régimes climatiques tant maritimes que continentaux; cependant, seuls les lacs dont les bassins sont sur des substrats précambriens cristallins ont été retenus, de façon à éliminer les effets édaphiques du roc sédimentaire alcalin. Une analyse des correspondances canoniques qui emploie la sélection ascendante et des tests de permutation de Monte Carlo a retenu le pH, la conductivité, la température estivale de l'eau du lac et la température moyenne annuelle de l'air comme les variables significatives du milieu qui expliquent le mieux les associations de diatomées. À l'aide de régressions et de calibrages basées sur les moyennes pondérées, nous avons élaboré des modèles prédictifs pour ces variables. Le modèle de la température estivale de l'eau du lac appliqué aux associations de diatomées qui se succèdent en profondeur dans les carottes de sédiments fournit des reconstitutions réalistes des températures par comparaison aux méthodes basées sur d'autres variables paléoenvironnementales. Au cours des 5 000 dernières années, l'amplitude des températures estivales reconstituées de l'eau du lac est de l'ordre de 4°C, ce qui représente principalement un refroidissement néo-glaciaire qui atteint son maximum durant la petite ère glaciaire. Les températures estivales de l'eau inférées à partir des diatomées ont augmenté de 2,0°C depuis 150 ans, ce qui correspond aux valeurs obtenues indépendamment dans d'autres reconstitutions paléoclimatiques.

[Traduit par la Rédaction]

Introduction

The Canadian Arctic Archipelago, of which Baffin Island represents the largest landmass (507 450 km^2), is highly susceptible to climatic variability on interannual to millennial timescales (Overpeck et al. 1997). Within the global climate system, the importance of the Arctic lies with its intimate linkages to ocean circulation, atmospheric feedbacks involving snow and ice cover, and biospheric modulation of greenhouse gas concentrations. The understanding of past climate trends in the Arctic serves as an important component of the ability to predict future changes, which will likely manifest themselves as complex synergisms between anthropogenic forcing and natural variability. At the same time, due to a sparse human population, Arctic ecosystems are as close to pristine as anywhere on earth.

Lake sediments are attractive repositories for paleoclimate proxy data because they are temporally continuous, undisturbed, and datable. Furthermore, lakes are ubiquitous

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throughout most of the Arctic, enabling relatively dense spatial coverage of sampling sites. Although the pollen record from Baffin Island sediments has provided useful paleoenvironmental inferences (Andrews et al. 1981), low pollen production by local plants, frequently coupled to overrepresentation by extraregional pollen, hampers direct interpretations in terms of regional vegetational and paleoclimatic history. In contrast, diatoms have greatly simplified depositional trajectories to lake sediments, where their siliceous microfossils are generally very abundant (e.g., $>10^6$ valves·cm^3 sediment^{-1}), well preserved, and identifiable. In an evolving body of literature, inference models have been developed for the use of diatoms as quantitative paleoclimatic indicators (Pienitz et al. 1995; Weckström et al. 1997; Rosén et al. 2000). Although the exact ecophysiological relationships between diatom communities and temperature remain uncertain, if they exist at all, it is likely that the success of these approaches relies on the indirect linkages between climate and limnological parameters that control algal populations (Smol 1988).

Given the size of Baffin Island and the range of aquatic habitats present, diatoms have historically received relatively little attention (Ross 1947; Moore 1975). More recently, diatoms have been applied to a multitude of paleoenvironmental questions (Wolfe 1996*a*, 1996*b*). However, these studies have been limited by the absence of a regional training set to enable quantitative paleoenvironmental inferences. The training set ideally represents a network of modern samples and associated environmental data that encompass the full breadth of floristic variability encountered in the fossil diatom record and, by association, of past environmental conditions. This inferential tool and an example of its application are provided by this study. Ordination, regression, and calibration of diatom assemblages from 61 Baffin Island lakes indicate that limnological (pH and conductivity) and climatic (summer water and mean annual temperatures) parameters exert significant influences on floristic composition and therefore can be reconstructed from the fossil record of this region. These data have implications for both the ecology of Arctic freshwater diatom communities and the reconstruction of regional paleoclimatic conditions.

Regional background and study sites

Baffin Island occupies the southeastern Canadian Arctic Archipelago between 62 and 74°N (Fig. 1). As the northeastern margin of the Canadian Shield, bedrock geology is primarily Precambrian granite, gneiss, and quartz monzonite, although Paleozoic carbonates comprise much of the western lowlands surrounding Foxe Basin (Jackson and Taylor 1972). The climate of Baffin Island is controlled by a trough in circumpolar westerly flow (the Baffin Trough) as well as by heat transport from the North Atlantic via the Labrador Sea and Baffin Bay (Keen 1980). As a consequence, precipitation decreases sharply from the maritime east coast to the more continental western portion of the island (Fig. 2*a*). In contrast, the temperature gradient is roughly latitudinal (Fig. 2*b*). The vegetation of Baffin Island is classified into three broad zones (Fig. 1): low Arctic in the south (dwarf shrub sedge – moss – lichen), transitional mid-Arctic (stony heath sedge – moss – lichen), and high Arctic (rock desert) in the north (Porsild 1964).

Lakes selected for this study are situated exclusively on crystalline terrains and thus are not influenced by carbonates, which translate to elevated lake water pH. This sampling design deliberately aims to maximize the climatic gradient reflected in the sediments by minimizing contrasting edaphic gradients created by the higher pH of carbonate-basin lakes in the western part of the region. The lakes are distributed across the climatic gradients of precipitation and temperature and distributed in each of the three major vegetation zones (Fig. 1). In order to eliminate the potential impact of lake ice on the integrity of bottom sediments, lakes with a maximum water depth <3 m were eliminated from the data set. The selected lakes are therefore morphometrically comparable with those chosen for coring and paleoenvironmental reconstructions. This is an important consideration in the attempt to provide adequate modern biological analogs for past conditions as reflected by core assemblages.

Materials and methods

Modern sediment sample collection and preparation

During three successive summer field seasons (between late July and mid-August of 1994, 1995, and 1996), surface sediment and water samples were collected from Baffin Island lakes during helicopter surveys. All sampling was performed from an inflatable boat. At each lake, the deepest point was identified with a digital depth finder. From this location, sediment monoliths preserving the mud–water interface intact were collected with a miniature Ekman dredge. From the dredge material, the top 1.0 cm was carefully removed with a spatula and stored at 4°C until analysis. Lyophilized subsamples (0.10–0.20 g dry mass) were prepared for diatom analysis by oxidation in 30% H_2O_2 (30 min) and thereafter boiled in concentrated (93%) HNO_3. Once cooled, samples were centrifuged, washed, and rinsed with deionized water at least four times and then diluted to a volume of 10 mL. Aliquots of 0.20 mL were removed from this slurry, diluted by a factor of 50, and homogenized with a vortex agitator. Drops of this second slurry were pipetted onto coverslips, evaporated at room temperature, and mounted permanently to slides with Naphrax® medium. From each slide, 400–500 diatom valves were identified and enumerated along random transects including coverslip edges at 1000× under oil immersion objectives on an Olympus Vanox® microscope equipped with Hoffman modulation optics. Once tallied, raw counts were converted to relative frequencies, that is, the proportion of individual taxa relative to the total diatom count in the sample, expressed as a percentage. All numerical analyses were preformed on the relative frequency data. Principal taxonomic references for diatom identification include Patrick and Reimer (1966, 1975), Foged (1981), and Krammer and Lange-Bertalot (1986–1991). The names of several genera, particularly within *Navicula* and *Fragilaria* sensu lato, have been revised according to the proposals of Round et al. (1990). A complete voucher set of slides has been permanently deposited in the diatom herbarium at the Canadian Museum of Nature, Ottawa, Ont.

Environmental data

Several limnological measurements were made in the field with sensors deployed on a SeaCat® profiler: conductivity, water temperature, maximum water depth (measured as pressure), and transmissivity (25-cm light path at 660 nm). Because of the extremely low ionic strength of the sampled waters, field pH measurements were deemed unreliable due to insufficient time for

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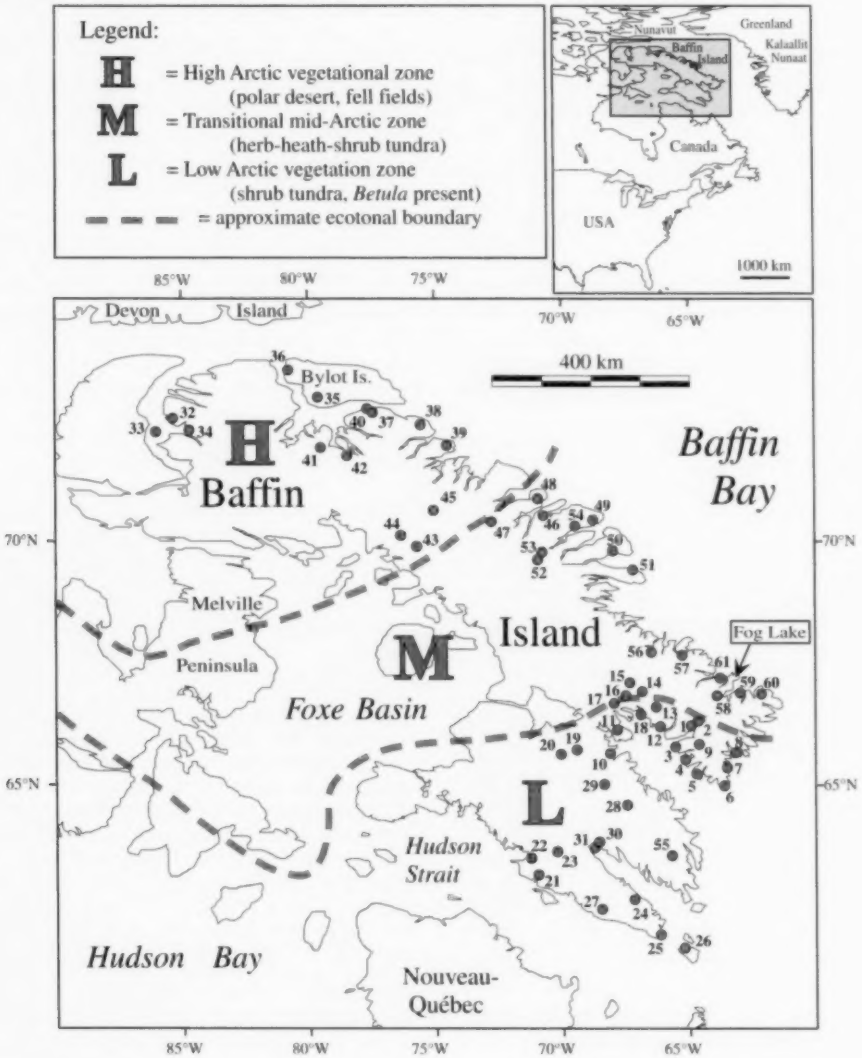
Fig. 1. Map of Baffin Island showing the locations of the 61 lakes used to develop the diatom inference models. Approximate positions of major ecotonal boundaries are also shown (Porsild 1964), as is the location of Fog Lake, the site used to test the summer water temperature inference model on down-core assemblages.



adequate electrode equilibration during SeaCat casts. Therefore, the pH data used here are from subsequent laboratory measurements. For conductivity, the upper 2 m of casts were averaged to provide a representative epilimnetic value, thus avoiding the potential influence of seasonal solute concentration in the hypolimnion. Downcast averages of the whole water column were used for both transmissivity and water temperature. In the case of water temperature, this approach was used for three reasons: (*i*) to minimize time of day sampling artifacts, (*ii*) to integrate whole-lake thermal inertia and minimize time of season differences, and (*iii*) to capture deep-water environments inhabited by many of the benthic diatom taxa.

Sampling position (latitude and longitude) and altitude were determined from the helicopter's global positioning system receiver and altimeter (±5 m), respectively. Lake surface and watershed areas were determined from areal photographs (1 : 60 000) and topographic maps (1 : 50 000) using either a planimeter or digital image analysis. The climatic data (Fig. 2) are based on a 25-km gridded climatology for North America (Thompson et al. 1999). Mean annual air temperature, mean January air temperature, mean July air temperature, annual precipitation, January precipitation, and July precipitation were calculated for each of the 61 sites by averaging the four nearest-neighbor grid points of the 25-km grid. Given the remoteness of Baffin Island and the overall paucity of both limnological and climatic data for the entire region, the raw environmental data are reported in their entirety (Appendix).

Fossil samples

The performance of the summer water temperature inference model was tested on fossil assemblages from a short core (34.5 cm) from Fog Lake (67°11′N, 63°15′W) on northern Cumberland Peninsula. This lake, described in detail elsewhere (Wolfe

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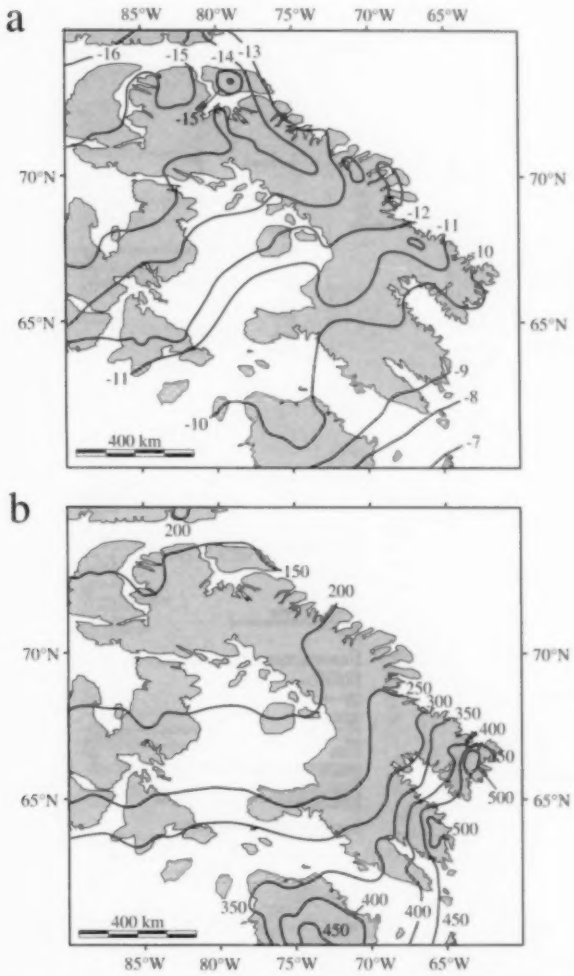
Fig. 2. Climatic characterization of the study area based on the 25-km-gridded climatology of North America (Thompson et al. 1999). (*a*) Mean annual temperature (°C); (*b*) mean annual precipitation (mm H_2O equivalent).



et al. 2000), lies approximately midway on the latitudinal transect of the training set sites (Fig. 1). This implies that the range of past environmental conditions at Fog Lake have realistic analogs from within the 61-lake modern training set. The core was collected in 1997 with a modified K-B gravity corer (Glew 1989) and subsampled into continuous 0.5-cm increments in the field. Diatoms were prepared and counted from each interval in the same way as the modern samples. The core's chronology is based on five accelerator mass spectrometry (AMS) ^{14}C dates from sediment humic acids extracted according to Abbott and Stafford (1996). The humic acid ^{14}C ages were corrected by adding 300 years to account for the average lag associated with the sequestration of terrestrial dissolved organic carbon to lake sediments (Miller et al. 1999) and then calibrated to calendar years before present using the CALIB software package (Stuiver and Reimer 1993).

Numerical techniques

Three ordination techniques were employed, first to explore the structure of the environmental and biological data sets taken independently and thereafter to evaluate the diatom data when constrained to the environmental data. Generally speaking, ordination is a family of multivariate techniques that reduce the dimensionality of complex data to a few interpretable gradients (or axes) that constitute synthetic variables accounting for the major patterns of variation within the data (ter Braak 1987). Ordinations were implemented with the software CANOCO 3.11 (ter Braak 1988–1992). Principal components analysis (PCA) was used to identify and summarize patterns within the environmental data. PCA is a linear indirect ordination technique that gives a low-dimensional representation of multivariate data, which can be examined visually to identify structure. PCA, using a centered correlation matrix, is appropriate for interval data and relatively short gradients, such as the various environmental variables available for each of the 61 sites considered here. Detrended correspondence analysis (DCA) (Hill and Gauch 1980) was used as a diagnostic tool to evaluate the length of gradients expressed by the diatom data and to summarize major patterns of variation within the diatom assemblage data. DCA is a unimodal method of indirect ordination that reports gradient lengths in standard deviation units and is particularly useful for determining whether linear or unimodal techniques are most appropriate for the data under consideration (ter Braak and Prentice 1988). Finally, canonical correspondence analysis (CCA) (ter Braak 1986), a direct (or constrained) unimodal ordination technique, was applied to the combined environmental and diatom data sets in order to evaluate the former's performance as a predictor of the biological response reflected by diatom taxonomic assemblages. The axes in CCA ordination are constructed as linear combinations of the environmental variables (ter Braak 1987). CCA can thus be used to identify the environmental parameters that directly account for significant and independent variations in the species data. Forward selection of individual environmental variables, followed by Monte Carlo permutation tests (999 permutations), was used to assess the statistical significance of specific environmental variables over diatom assemblage composition (Hall and Smol 1992; Pienitz et al. 1995).

Diatom-based inference models were developed for each of the significant environmental variables using weighted-averaging (WA) regression and calibration. WA assumes that a given diatom taxon is most abundant in lakes where a significant environmental parameter is at, or near, this taxon's ecological optimum. With this assumption, WA uses the weighted abundances of taxa throughout the modern training set to determine each taxon's optimum and tolerance by regressing taxon relative frequencies with regards to the environmental data (Birks 1995). Once optima and tolerances for all taxa are defined, environmental inferences can be predicted from diatom assemblages (calibration), whether a corresponding series of environmental measurements exists (the modern training set samples) or not (fossil samples). In WA, averages are taken twice, once during the regression step and again during calibration. This causes an artifact within diatom-inferred values referred to as shrinkage, which can be corrected by either classical or inverse deshrinking methods (Birks 1995). Although inverse deshrinking generally produces lower root mean square errors (RMSE) of prediction, it typically underestimates values at the high end of a parameter's range and overestimates values at the gradient's low end (Pienitz et al. 1995). Classical deshrinking, despite generally resulting in higher apparent RMSE, does not produce the trends in residuals produced by inverse deshrinking and therefore was considered more reliable for the present study. All WA calibration calculations were performed using WACALIB 3.3 (Line et al. 1994). This program also calculates bootstrapped RMSE estimates, provided by a computationally intensive resampling procedure in which a new training set equal in size to the original is generated by random selection with replacement from within the original data, for 1000 iterations.

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Fig. 3. Relative frequencies of the 36 most abundant diatoms in the 61 study lakes. The sites are arranged by order of downward-increasing measured summer lake water temperature, whereas the taxa are presented in order of decreasing WA water temperature optima (Table 1).

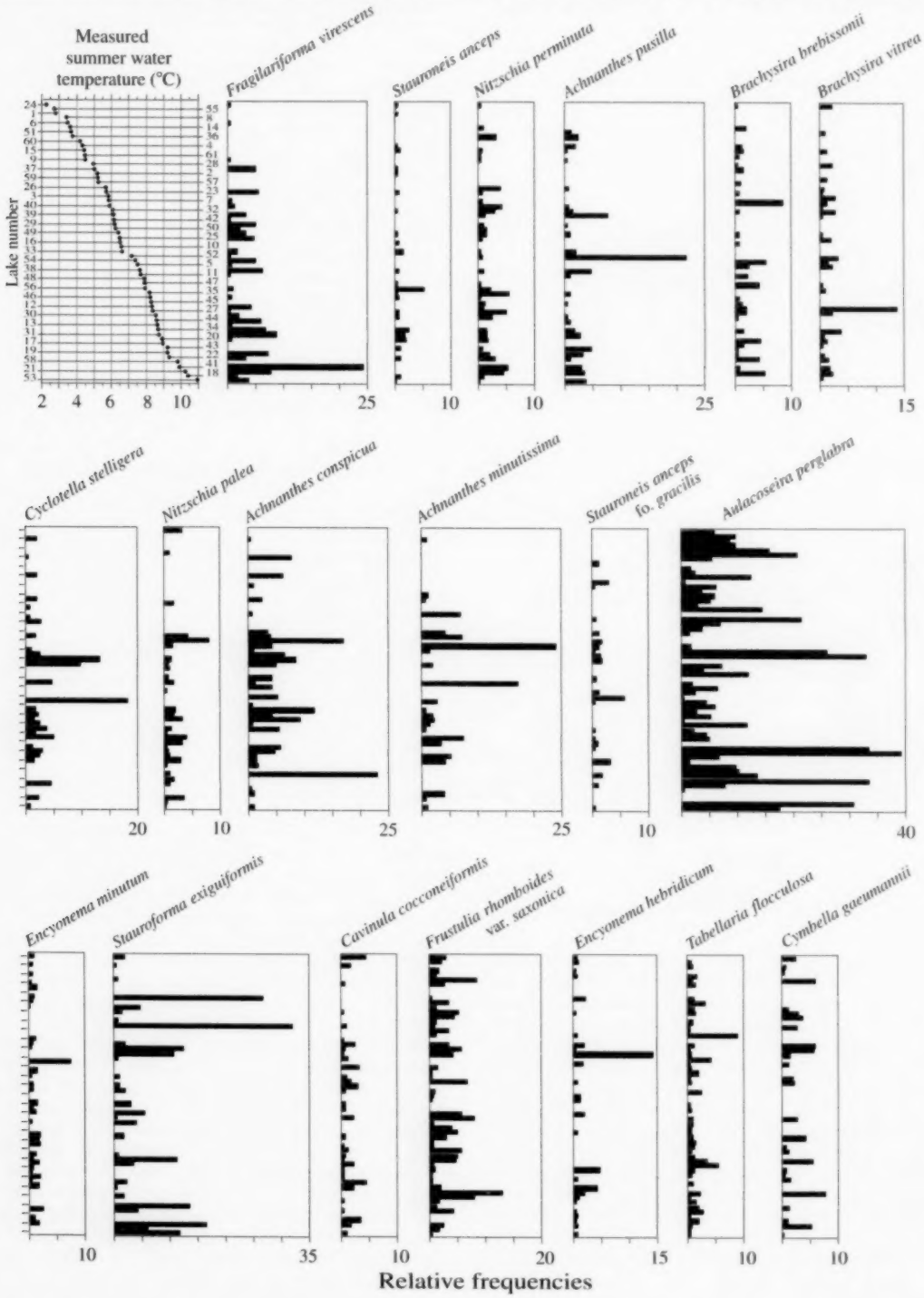

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Fig. 3 (*concluded*).

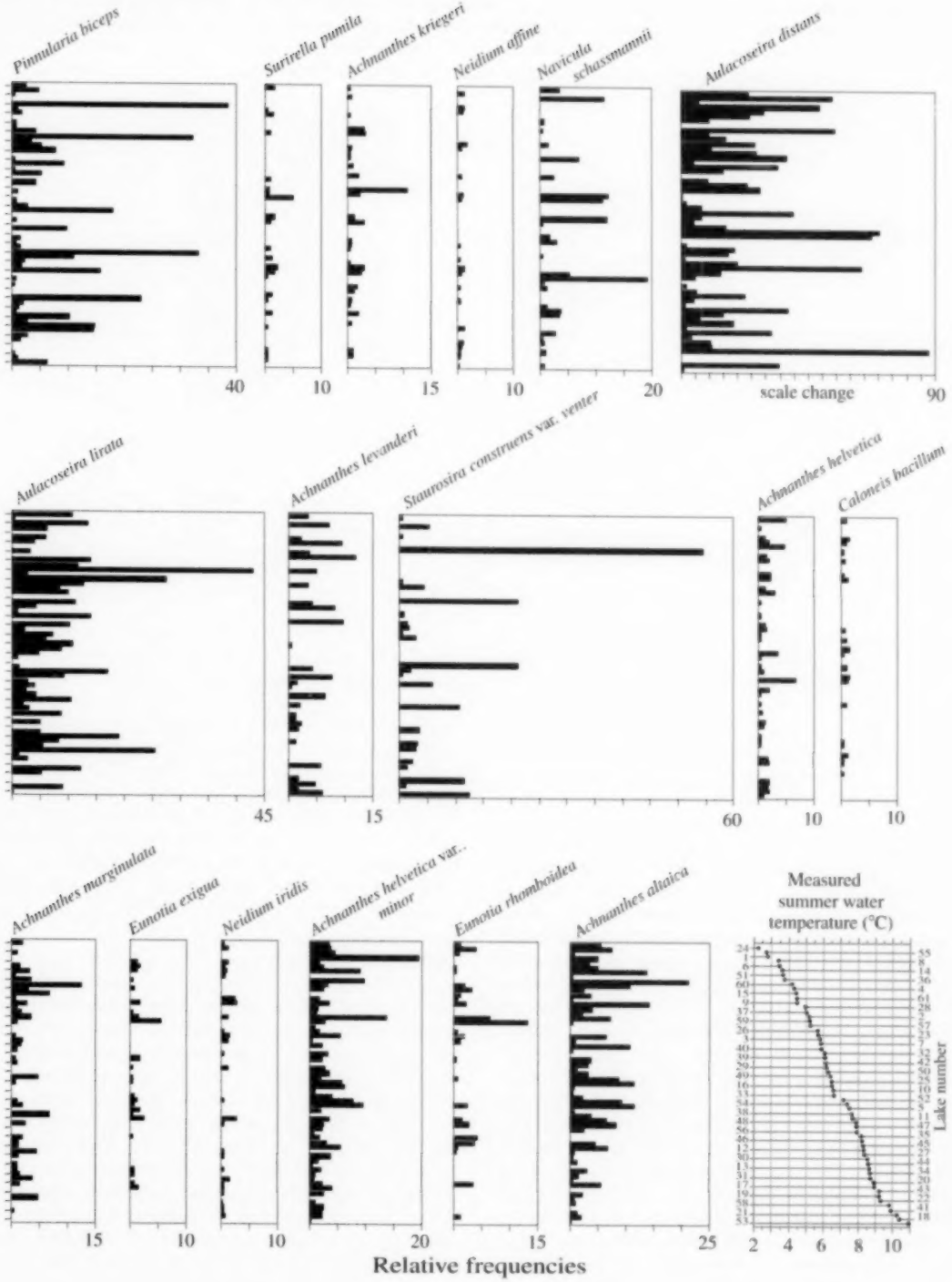

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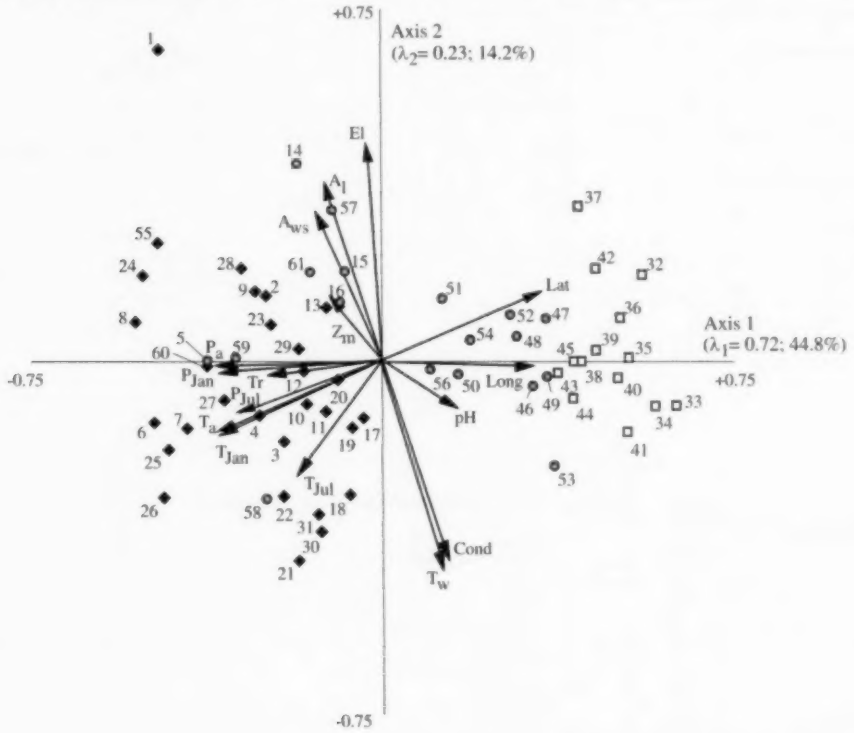
Fig. 4. PCA correlation biplot showing site scores and loadings of the environmental variables (arrows) from the 61 sampled lakes. Lat, latitude; Long, longitude; El, elevation; Z_m, maximum water depth; A_l, lake surface area; A_{ws}, catchment area; pH, lake water pH; Cond, conductivity; Tr, transmissivity; T_w, summer lake water temperature; $T_{(a, Jan, Jul)}$, mean air temperature (annual, January, July); $P_{(a, Jan, Jul)}$, precipitation (annual, January, July). Symbols are used to differentiate lakes from the different ecological zones as follows: High Arctic (open squares), transitional mid-Arctic (gray circles), and low Arctic (solid diamonds).



Results

General floristic description

A total of 234 diatom taxa were identified, representing 48 genera. Of these, 107 taxa from 32 genera have relative frequencies >1% in at least three samples, and only these were retained for numerical analysis. The complete taxonomic inventory with authorities is presented elsewhere (Joynt 1999). A maximum of 65 taxa were recorded in any one lake (Lake 21); the fewest taxa identified were 12 (Lake 10). Relative frequencies of the 36 most common diatom taxa, defined as having effective occurrence values (Hill's N2) >15, are illustrated in Fig. 3. The diatom flora is typical of dilute, acid-sensitive lakes of the northern Canadian Shield (Clark et al. 1989; Grönlund et al. 1990) but differs markedly from the floras of shallow polar ponds (Douglas and Smol 1993). Although planktonic diatom diversity is low (limited to several species of *Aulacoseira* and *Cyclotella*), planktonic forms are present at every site. In fact, *Aulacoseira distans*, *A. perglabra*, and *A. lirata* are consistently well represented in the modern samples (Fig. 3). Nonetheless, the benthic flora has far greater taxonomic richness, and this likely reflects a combination of deep light penetration, diverse habitats for periphytic growth forms, and the brevity of the open-water season available for phytoplankton growth (Lotter and Bigler 2000). The benthic flora is primarily dominated by small *Achnanthes* species (*A. altaica*, *A. helvetica*, *A. helvetica* var. *minor*, *A. kriegeri*, *A levanderi*, *A. marginulata*, *A. minutissima*, and *A. pusilla*), *Pinnularia biceps*, as well as several small fragilarioid diatoms (*Fragilariaforma virescens*, *Stauroforma exiguiformis*, and *Staurosira construens* var. *venter*).

Ordination experiments

The PCA of the environmental data is illustrated by a correlation biplot (Fig. 4), where arrows represent the environmental variables and the angles between their respective vectors reflect the varying degree of collinearity between them. The arrows point towards increasing values of a given parameter, whereas arrow length corresponds to the length of the environmental gradient. The first two PCA axes accounted for 59.0% of the variation within this data, with eigenvalues of $\lambda_1 = 0.72$ (44.8% of inertia) and $\lambda_2 = 0.23$ (14.2%). Eigenvalues for subsequent axes are much lower ($\lambda_3 = 0.16$ and $\lambda_4 = 0.12$) and these are not dealt with further. The relationships illustrated by the PCA biplot are entirely consistent with the climatology and physiography of the study area. For example, the latitudinal gradient increases in the opposite direction from the air temperature parameters, whereas longitude is an anticorrelate of the precipitation parameters. This is in agreement with the region's climatic regime and attests to the integrity of the

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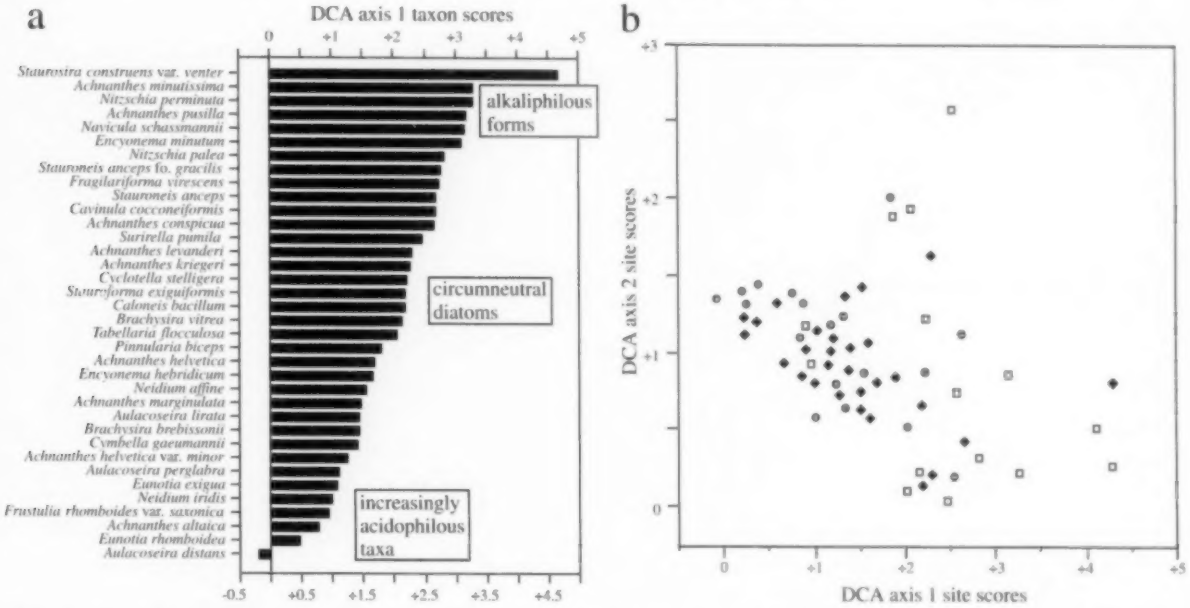
Fig. 5. (*a*) Species scores for the 36 most abundant diatoms (cf. Fig. 3) on the first axis of DCA ordination and (*b*) joint plot of corresponding sample scores along the first two axes of the same ordination. Symbols are used to differentiate lakes from the different ecological zones as follows: High Arctic (open squares), transitional mid-Arctic (gray circles), and low Arctic (solid diamonds).



gridded climatic data (Fig. 2). The summer water temperature gradient is opposite to those of maximum depth, lake surface area, and elevation. This implies that lakes that are shallower, smaller, and at lower elevations attain the greatest summer temperatures, regardless of their latitude. Furthermore, the similarity between gradients of summer water temperature and conductivity implies that the warmer lakes undergo measurable evaporative solute enrichment during summer. The gradient of lake water pH combines the influences of ionic strength and temperature as well as longitude, which act as a surrogate for the westerly increase in the amount of carbonate in glacial drift.

The first two axes of the DCA performed on the diatom relative frequency data have eigenvalues of 0.58 and 0.30, respectively, representing 13.5 and 7.0% of the variance within the diatom relative frequency data. This translates to gradient lengths, in standard deviation units, of 4.41 for axis 1 and 2.56 for axis 2. For gradient lengths exceeding 2.0 standard deviation units, the data are best accommodated by unimodal response models, for which DCA and CCA are the most appropriate techniques (ter Braak and Prentice 1988). The relatively low percentage of variance explained by the first two DCA axes (20.5%) is not unusual for data characterized by a large number of taxa (107) and many zero entries (Birks 1995). Of equal importance to the proportions of variance explained by the first two axes is the overall stability of the ordination result itself, as indicated by the low value of the third axis eigenvalue ($\lambda_3 = 0.19$) relative to those of the first ($\lambda_1 = 0.58$) and second ($\lambda_2 = 0.30$) axes (ter Braak 1987). Furthermore, the DCA results are ecologically interpretable. For example, axis 1 scores for the 36 most abundant taxa (Fig. 5*a*) show a clear separation between alkaliphilous and acidophilous taxa, as documented by studies of similar floras addressing diatom–pH relationships explicitly (Dixit et al. 1999). In turn, DCA axis 1 site scores and measured pH are significantly correlated for the 61 sites ($r^2 = 0.67$, $p < 0.001$). This attests to the universality of pH as a primary control over the composition of diatom floras in dilute lakes (Battarbee et al. 1999). The ecological coherence of the 61 Baffin Island diatom assemblages is confirmed by the fact that DCA identifies the primary ecological gradient (pH) in the absence of any a priori ecological assignments to the individual taxa. However, because the DCA results appear more intimately related to limnological rather than climatic factors, there is no clear separation of lakes from the three climatic zones in the joint plot of site scores along the first two DCA axes (Fig. 5*b*). To summarize the results of the indirect ordinations of the environmental (PCA) and diatom (DCA) data sets, it is apparent that both have internally coherent and interpretable structures; this raises confidence in the ability to derive meaningful inference models from the two sets of data when combined.

The preliminary CCA ordination constrained the 61 diatom assemblages to all 16 limnological and climatic variables. The first two axes collectively explain 14.1% of the variance in the diatom assemblage data ($\lambda_1 = 0.43$ (10.1%), $\lambda_2 = 0.17$ (4.0%)). The species–environment correlation was 0.88 for CCA axis 1 and 0.79 for CCA axis 2, which translates to a total of 41.8% of the diatom–environment relationship being explained (30.2 and 11.6% for each of axes 1 and 2, respectively). This demonstrates a strong relationship between the diatom assemblages and measured environmental and climatic parameters. Unrestricted Monte Carlo permutation tests performed on these two axes show both to be statistically significant ($p < 0.01$).

Using forward selection and further unrestricted permuta-

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Fig. 6. CCA biplots of (*a*) all environmental variables (thick arrows indicate forward selection), (*b*) species scores, and (*c*) site scores. The names of the 107 diatom taxa are given in Table 1. Information for the sites is given in the Appendix. See Fig. 4 for abbreviations. In Figs. 6*b* and 6*c*, the four significant environmental variables are reproduced.

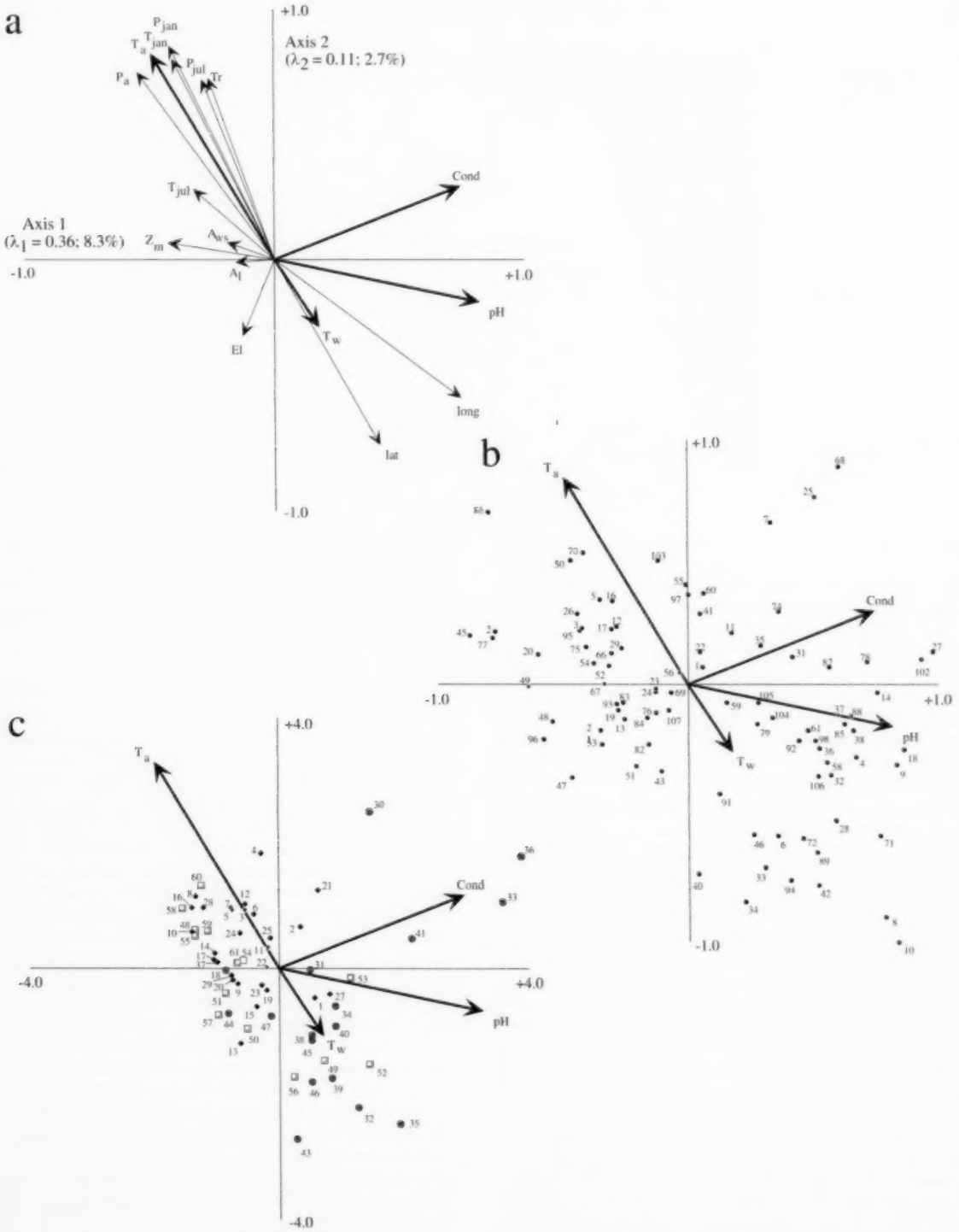

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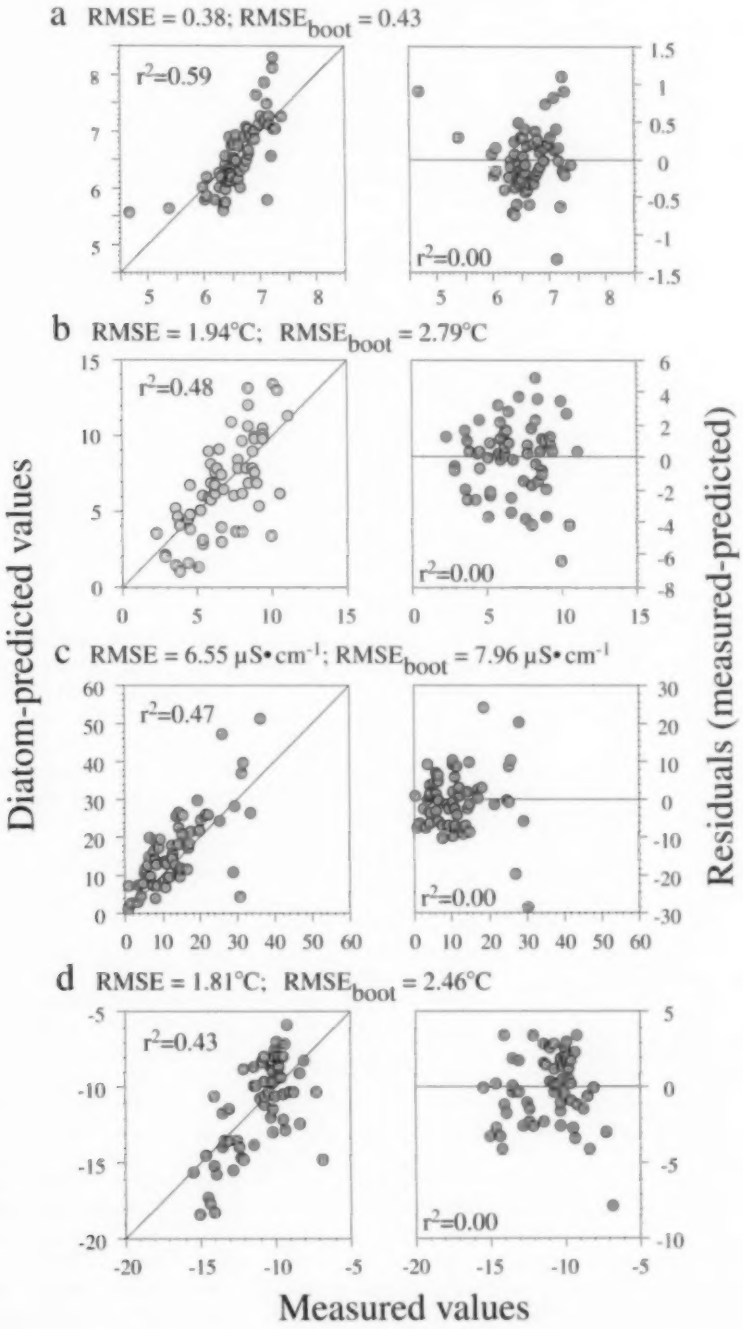
Fig. 7. Relationships between measured environmental parameters and their diatom-inferred counterparts for the 61 study lakes. (*a*) Lake water pH; (*b*) summer temperature; (*c*) conductivity; (*d*) mean annual air temperature. The relationships are all significant at $p < 0.05$. Plots on the right depict the residuals for the same parameters.



tion tests, four environmental variables emerged as accounting for significant ($p < 0.05$) proportions of variance within the diatom assemblage data. These four variables collectively account for 42.2% of the variance initially explained by the original 16 variables for the first two CCA axes. Significant variables include pH (16.9%), conductivity (9.2%), summer lake water temperature (8.1%), and mean annual air temperature (8.0%). When forward selected as the only

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Table 1. WA optima (Opt.) and tolerances (Tol.) for pH, conductivity (Cond.), summer water temperature (SWT), and mean annual air temperature (MAT) for the 107 taxa retained in the WA calibration and regression.

	pH		Cond.		SWT		MAT			
Taxon	Opt.	Tol.	Opt.	Tol.	Opt.	Tol.	Opt.	Tol.	Occ.	Hill's N2
1. *Achnanthes lanceolata*	6.74	0.38	9.64	8.43	5.87	2.35	−10.73	2.19	11	7.2
2. *Achnanthes calcar*	6.20	0.73	9.37	6.33	6.08	2.55	−10.62	1.88	7	5.3
3. *Achnanthes clevei* var. *bottnica*	6.45	0.25	8.32	3.25	6.00	2.00	−10.39	1.45	8	6.3
4. *Achnanthes delicatula*	6.76	0.36	13.83	8.18	6.92	2.01	−12.24	1.79	15	10.4
5. ***Achnanthes marginulata***	6.50	0.40	9.49	6.14	6.28	2.04	−10.40	1.77	39	28.2
6. ***Achnanthes conspicua***	6.75	0.35	12.95	6.29	7.11	1.65	−12.28	2.03	33	25.6
7. *Achnanthes flexella*	6.76	0.38	15.01	9.45	7.63	2.16	−10.14	2.14	15	9.0
8. *Achnanthes suchlandtii*	6.85	0.31	12.54	6.96	6.83	2.33	−12.46	2.94	7	4.2
9. ***Achnanthes pusilla***	6.88	0.30	15.98	6.48	7.60	2.06	−12.24	2.12	27	18.7
10. *Achnanthes pusilla* var. *petersenii*	6.91	0.22	17.52	9.80	6.44	1.03	−13.49	2.62	9	3.9
11. ***Achnanthes levanderi***	6.65	0.41	12.22	7.41	6.56	2.51	−11.01	2.03	28	23.5
12. ***Achnanthes altaica***	6.49	0.46	10.18	7.25	6.14	2.05	−10.84	1.83	53	38.8
13. *Achnanthes gracillima*	6.55	0.31	10.71	8.06	6.59	2.53	−11.10	2.11	8	5.3
14. ***Achnanthes krïegeri***	6.75	0.33	13.16	8.41	6.65	1.83	−11.73	2.28	28	19.3
15. *Achnanthes saccula*	6.86	0.55	17.54	17.27	4.45	2.07	−8.38	2.40	2	2.0
16. ***Achnanthes helvetica***	6.48	0.38	10.28	6.69	6.44	2.45	−10.54	1.99	38	26.9
17. ***Achnanthes helvetica* var. *minor***	6.52	0.44	10.69	7.70	6.35	2.16	−10.91	1.84	54	44.5
18. ***Achnanthes minutissima***	6.91	0.25	15.92	7.53	7.34	1.58	−11.92	2.80	23	17.2
19. ***Aulacoseira lirata***	6.51	0.42	9.75	6.99	6.50	2.05	−11.03	1.80	50	42.7
20. ***Aulacoseira distans***	6.43	0.49	9.25	6.77	6.40	2.17	−10.71	1.60	55	46.2
21. ***Aulacoseira perglabra***	6.52	0.33	9.18	6.22	6.69	2.29	−10.90	1.63	52	43.2
22. ***Brachysira vitrea***	6.65	0.33	12.73	7.51	7.16	2.02	−10.70	2.29	30	21.6
23. ***Brachysira brebissonii***	6.62	0.29	10.49	5.69	7.36	1.94	−10.95	1.78	30	21.6
24. ***Caloneis bacillum***	6.52	0.37	10.10	6.26	6.19	2.06	−11.14	1.77	21	17.5
25. *Caloneis holstii*	6.86	0.06	19.42	6.40	9.96	0.54	−9.87	2.47	3	2.4
26. *Craticula accomoda*	6.30	0.76	11.37	9.18	6.41	2.06	−10.76	1.83	16	12.5
27. *Cymbella naviculiformis*	6.96	0.29	18.62	6.18	7.98	2.31	−11.62	2.49	9	7.1
28. *Cymbella cesatii*	6.90	0.25	14.37	7.69	8.15	1.38	−12.28	2.18	13	10.8
29. ***Cymbella gaeumannii***	6.54	0.31	9.84	7.60	6.64	2.22	−10.61	1.54	27	20.8
30. *Cymbella angustata*	7.01	0.26	19.04	8.87	7.50	1.88	−11.48	2.82	9	6.9
31. ***Cavinula cocconeiformis***	6.76	0.32	14.06	7.56	6.99	2.28	−11.22	2.23	34	24.6
32. *Cavinula jaernefeltii*	6.80	0.44	14.78	8.21	8.29	2.07	−11.92	1.98	11	8.6
33. *Cavinula pseudoscutiformis*	6.79	0.35	12.22	6.25	7.68	1.91	−12.22	1.48	19	13.1
34. *Cavinula variostriata*	6.61	0.35	7.51	5.32	5.74	2.15	−11.87	2.64	13	7.6
35. ***Cyclotella stelligera***	6.76	0.29	12.59	5.74	7.32	1.66	−11.06	2.10	28	21.9
36. *Cyclotella rossii*	6.82	0.33	16.89	6.99	7.94	1.63	−11.96	2.03	22	12.1
37. *Cyclotella bodanica* var. *lemanica*	6.83	0.28	15.21	5.77	7.28	1.70	−11.56	2.38	19	14.6
38. *Cyclotella tripartita*	6.84	0.22	17.30	8.57	7.80	1.78	−11.83	2.57	10	6.4
39. *Denticula tenuis*	7.16	0.20	27.15	2.02	7.24	3.10	−9.07	4.42	3	2.0
40. *Diploneis oculata*	6.70	0.25	12.35	10.00	8.26	1.33	−12.43	1.78	5	3.5
41. *Eunotia pectinalis*	6.52	0.61	13.22	8.33	7.08	1.67	−10.85	2.43	19	14.4
42. *Eunotia pectinalis* var. *minor*	6.77	0.30	12.79	6.69	6.71	1.87	−12.63	1.91	17	9.8
43. *Eunotia praerupta*	6.67	0.32	10.56	5.34	7.48	2.04	−11.31	1.88	18	10.4
44. *Eunotia praerupta* var. *bidens*	7.07	0.33	19.15	8.56	9.10	1.90	−11.18	2.22	6	4.7
45. *Eunotia bidentula*	6.38	0.40	4.33	4.78	4.78	2.47	−9.91	0.84	8	5.6
46. *Eunotia monodon*	6.66	0.28	12.39	3.15	6.91	1.23	−12.68	2.22	5	4.1
47. ***Eunotia exigua***	6.43	0.36	8.37	5.53	6.25	1.84	−11.38	1.77	24	17.9
48. ***Eunotia rhomboidea***	6.49	0.34	7.53	6.61	6.04	2.11	−10.67	1.64	26	18.7
49. *Eunotia denticulata*	6.44	0.26	5.40	3.86	5.64	2.24	−10.27	0.83	12	7.9
50. *Eunotia incisa*	6.45	0.28	8.13	7.14	5.32	2.26	−9.91	0.74	11	7.7
51. *Eunotia bilunaris*	6.54	0.40	9.69	8.70	6.55	1.98	−11.69	2.05	19	12.8
52. *Eunotia vanheurckii*	6.49	0.29	10.16	4.99	6.97	2.13	−10.47	1.89	18	13.9
53. *Eunotia bilunaris* var. *mucophila*	6.61	0.36	6.34	5.67	5.88	2.44	−10.87	1.42	9	6.9
54. *Eunotia rhyncocephela*	6.63	0.43	5.55	7.25	5.02	2.65	−10.22	1.45	7	4.9
55. ***Encyonema hebridicum***	6.59	0.47	11.91	7.68	6.93	2.13	−10.52	1.56	27	17.1

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Table 1 (*concluded*).

Taxon	pH		Cond.		SWT		MAT		Occ.	Hill's N2
	Opt.	Tol.	Opt.	Tol.	Opt.	Tol.	Opt.	Tol.		
56. *Encyonema brehmii*	6.56	0.16	11.61	8.11	6.64	2.52	−10.98	1.03	3	2.5
57. *Encyonema latens*	6.96	0.16	20.98	8.33	8.23	2.39	−11.73	2.88	6	4.5
58. ***Encyonema minutum***	6.82	0.32	13.53	6.69	7.01	2.02	−11.66	2.61	34	25.8
59. *Encyonema lunatum*	6.74	0.25	12.67	6.41	7.85	1.92	−11.13	2.00	15	11.2
60. *Navicula vitrea*	6.55	0.29	10.56	6.28	5.62	2.43	−10.90	1.94	19	11.9
61. ***Fragilariforma virescens***	6.75	0.30	14.14	7.10	7.92	1.76	−11.43	2.15	35	24.8
62. *Staurosirella pinnata* var. *intercedens*	7.17	0.12	24.09	5.05	5.48	2.10	−11.40	4.48	4	3.1
63. *Pseudostaurosira brevistriata* var. *inflata*	6.98	0.26	18.02	8.62	6.67	2.21	−12.75	3.10	12	7.6
64. *Fragilaria vaucheriae*	6.85	0.22	17.33	9.01	7.14	1.89	−13.07	2.34	7	6.2
65. *Fragilaria vaucheriae* var. *capitellata*	6.92	0.19	19.92	8.79	8.02	1.96	−10.98	3.03	5	3.9
66. *Frustulia rhomboides*	6.57	0.28	10.76	7.70	7.72	2.04	−10.37	1.53	18	13.6
67. ***Frustulia rhomboides* var. *saxonica***	6.53	0.30	9.55	6.24	6.74	2.13	−10.70	1.52	50	37.3
68. *Frustulia rhomboides* var. *crassinervia*	6.87	0.05	20.61	5.71	10.07	0.50	−9.84	2.73	3	2.0
69. *Gomphonema parvulum*	6.69	0.18	10.08	7.58	6.94	2.89	−10.77	1.74	5	4.0
70. *Navicula mediocris*	6.39	0.35	8.86	3.94	5.54	2.61	−9.98	0.76	3	2.8
71. *Navicula bacillum*	6.99	0.24	14.71	7.70	7.89	1.29	−12.69	1.01	5	4.9
72. ***Navicula schassmannii***	6.76	0.32	11.43	6.15	6.61	2.15	−12.32	2.12	26	17.6
73. *Navicula paludosa*	7.09	0.47	24.07	10.98	7.14	2.91	−8.56	3.31	3	2.0
74. *Navicula radiosa* var. *tenella*	6.83	0.23	14.15	8.59	7.61	1.82	−10.64	2.63	14	11.1
75. ***Neidium iridis***	6.43	0.41	9.76	7.79	6.14	2.29	−10.64	1.54	28	19.3
76. ***Neidium affine***	6.59	0.56	10.05	7.82	6.62	2.49	−11.14	1.77	20	17.1
77. *Neidium dubium*	6.39	0.30	8.69	7.57	6.68	2.22	−9.97	1.61	11	7.4
78. ***Nitzschia perminuta***	6.84	0.31	15.21	7.81	7.52	1.95	−11.46	2.53	37	27.1
79. ***Nitzschia palea***	6.76	0.34	12.13	6.59	7.39	1.97	−11.42	2.47	30	22.4
80. *Nitzschia dissipata*	7.00	0.20	18.94	7.54	7.50	1.87	−12.71	3.28	9	7.0
81. *Nupela* sp.	6.57	0.13	11.82	4.41	6.59	1.27	−8.69	2.24	3	2.2
82. *Peronia fibula*	6.69	0.48	5.69	8.08	5.35	1.96	−10.92	1.78	7	5.4
83. *Pinnularia viridis*	6.58	0.27	8.96	4.80	6.94	1.83	−10.80	1.30	20	13.8
84. ***Pinnularia biceps***	6.57	0.39	9.63	6.23	6.69	2.18	−11.08	1.59	47	31.6
85. *Pinnularia biceps* var. *mesongyla*	6.89	0.34	15.85	9.36	7.53	2.35	−11.87	1.75	11	8.1
86. *Pinnularia intermedia*	6.32	0.30	3.65	1.57	3.60	1.40	−9.30	0.62	5	3.1
87. *Pinnularia brebissonii*	6.86	0.38	13.71	7.78	7.51	2.06	−11.16	1.23	14	10.0
88. *Pinnularia balfouriana*	6.81	0.38	16.15	8.47	7.35	2.10	−11.92	2.63	19	13.3
89. *Pseudostaurosira brevistriata*	6.80	0.39	10.38	8.88	5.17	2.63	−12.61	3.47	3	2.6
90. *Fragilaria pseudoconstruens*	7.04	0.25	21.23	6.81	7.29	2.24	−11.72	3.13	15	10.1
91. ***Stauroneis anceps***	6.75	0.30	11.65	6.73	7.65	1.84	−11.64	2.13	25	17.0
92. ***Stauroneis anceps* fo. *gracilis***	6.79	0.37	13.60	6.72	7.02	1.73	−11.82	1.95	22	16.3
93. *Stauroneis anceps* fo. *linearis*	6.59	0.31	8.31	4.97	6.45	2.05	−10.64	1.42	16	12.4
94. *Stauroneis phoenicenteron*	6.83	0.46	15.68	9.02	9.72	1.04	−12.24	1.80	5	3.5
95. *Stauroneis kriegeri*	6.51	0.38	9.40	8.44	6.31	2.46	−10.29	1.23	18	11.4
96. *Semiorbis hemicyclus*	6.60	0.23	9.31	6.98	9.11	0.70	−10.07	1.12	4	3.2
97. ***Stauroforma exiguiformis***	6.64	0.32	12.67	7.57	7.20	2.30	−10.66	1.77	31	21.8
98. *Sellaphora pupula*	6.87	0.34	14.05	8.48	8.12	1.46	−11.53	2.57	10	8.2
99. *Sellaphora laevissima*	6.79	0.53	17.10	12.18	6.78	3.49	−9.32	0.65	3	2.4
100. *Staurosira construens*	7.05	0.19	21.45	6.65	6.42	1.85	−12.52	3.65	11	6.1
101. ***Staurosira construens* var. *venter***	6.94	0.32	17.35	8.09	7.02	2.40	−12.24	2.63	25	16.5
102. *Staurosirella pinnata*	6.89	0.37	15.70	8.98	6.23	2.54	−11.99	2.55	20	13.8
103. *Surirella angusta*	6.76	0.26	10.95	5.06	7.79	2.42	−9.64	2.05	6	3.8
104. *Surirella ovalis*	6.63	0.39	12.93	8.54	6.41	1.75	−11.81	2.22	14	11.1
105. ***Surirella pumila***	6.73	0.31	12.37	6.58	6.63	2.07	−11.34	2.18	22	15.7
106. *Synedra ulna*	6.78	0.29	15.66	5.49	7.83	1.26	−12.10	2.51	10	6.1
107. ***Tabellaria flocculosa***	6.61	0.34	10.92	7.69	6.83	2.08	−11.23	2.05	49	33.0

Note: The number of occurrences (Occ.) and Hill's N2 index are also included. The 36 most frequent taxa for which the raw relative frequency data are shown in Fig. 3 are boldfaced.

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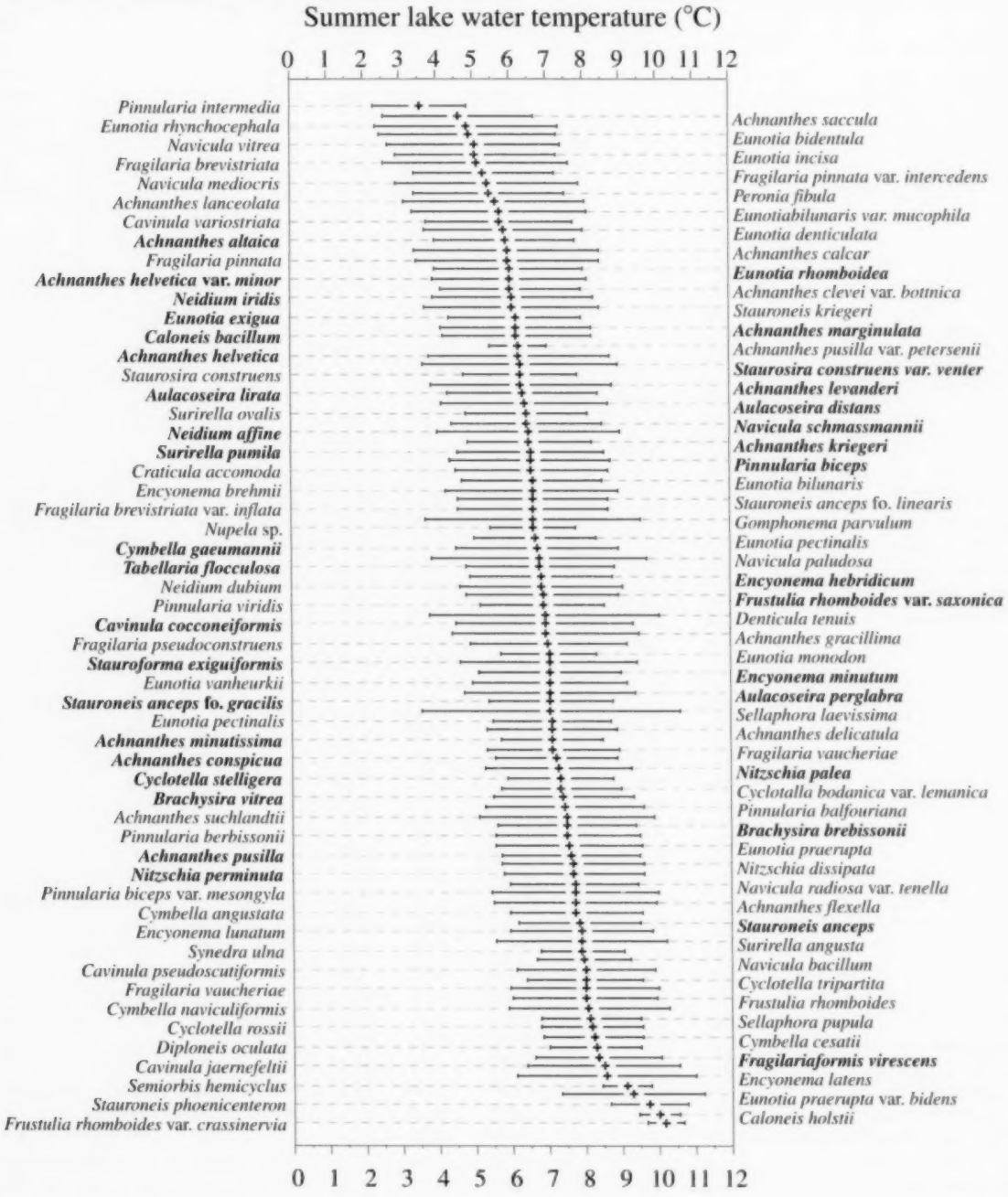
Fig. 8. WA optima (crosses) and tolerances (bars) for summer water temperature illustrated in ascending order for the 107 diatom taxa used to develop the model. The 36 most abundant taxa are boldfaced.



active variables in a second CCA, these four parameters account for 77.4% of the total variance in the diatom–environment relationship. No other individual variables explained significant additional proportions of variance within the diatom assemblage data. The CCA results are displayed to show the environmental gradients as well as the positions of the 107 taxa and the 61 lakes on the first two ordination axes (Fig. 6). The position of each taxon on the biplot is an approximation of its WA optimum relative to the other taxa. For example, taxa that commonly occur in high-pH and high-conductivity lakes are located on the positive (right) side of axis 1, whereas taxa associated with low-pH and more dilute waters are situated on the negative (left) side. Axis 2 is more strongly influenced by water and air tempera-

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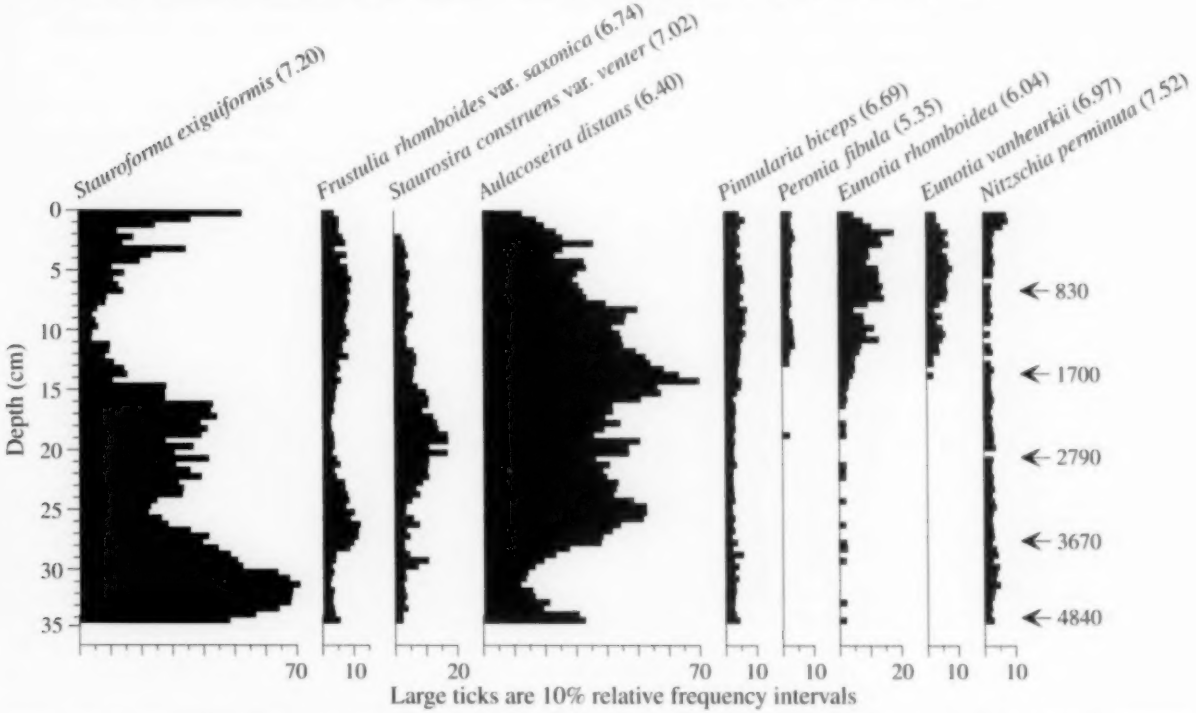
Fig. 9. Stratigraphy of dominant diatoms from the Fog Lake gravity core. The WA summer water temperature optimum is in parentheses next to each taxon. Numbers to the right are corrected and calibrated AMS ^{14}C dates in years before present.



tures, but a counterintuitive situation arises in which the gradients for annual air temperature and summer water temperature are almost opposite to each other (Fig. 6). This implies that measured summer water temperature is anticorrelated with the mean annual temperatures interpolated to each lake site. In reality, the water temperatures are unrelated to the gridded climatological data (temperature and precipitation), as shown by the orthogonal position of water temperature relative to these factors in the environmental PCA (Fig. 4). Physically, water temperature is dictated by lake radiation balance, which is sensitive to a variety of catchment-scale factors aside from ambient air temperature (Livingstone and Lotter 1998). This raises a certain amount of suspicion regarding the applicability for inference model development of air temperature data interpolated from a 25-km grid, despite the statistical significance borne out of the CCA. This suspicion is further supported by the fact that mean annual air temperature emerged as significant in the CCA, whereas July air temperature did not. The measured lake temperature values are clearly more strongly influenced by local edaphic factors, which may include shading, aspect, and ice cover (Lotter and Bigler 2000), as well as morphometric characteristics such as depth and area (Fig. 4). These local effects are well captured by the measured water temperature values but not by the interpolated air temperature data.

WA inference models

WA regression and calibration was undertaken for each of the four statistically significant environmental variables. When calibration is performed on the assemblages from the modern sediment samples, diatom-predicted values compare well with the actual measurements from the 61-lake network (Fig. 7). Importantly, the lack of any significant trends in the residuals suggests that simple WA with classical deshrinking is entirely appropriate for this data, as suggested by several other recent studies (Dixit et al. 1999; Gregory-Eaves et al. 1999). WA optima and tolerances for each of the 107 diatom taxa included in the training set are presented for the four significant environmental variables (Table 1).

Since the ultimate objective of this study is to develop a diatom-based inference model that is interpretable in paleoclimatic terms, and given the problems identified above concerning the applicability of the mean annual air temperature model, summer lake water temperature was the variable retained for additional study. The choice of this variable is further justified for the following reasons. Firstly, the primary data are measured and not interpolated. Secondly, summer water temperature captures the immediate environment in which diatoms grow as well as the timing of greatest production. Thirdly, summer water temperature has been shown to correspond linearly to locally measured air temperatures (Livingstone et al. 1999), despite the failure of July air temperature to emerge as significant in the present study. Finally, the independent paleoclimate proxies with which diatom-based inferences can be compared also reflect ice-free summer conditions. The summer water temperature optima and tolerances for all 107 taxa are shown in Fig. 8. This representation illustrates a great degree of continuity within the biological expression of the summer water temperature gradient, especially between 4.5 and 8.5°C. Although WA calibration enables extrapolation beyond the ranges of either species optima (3.6–10.1°C) or the site measurements them-

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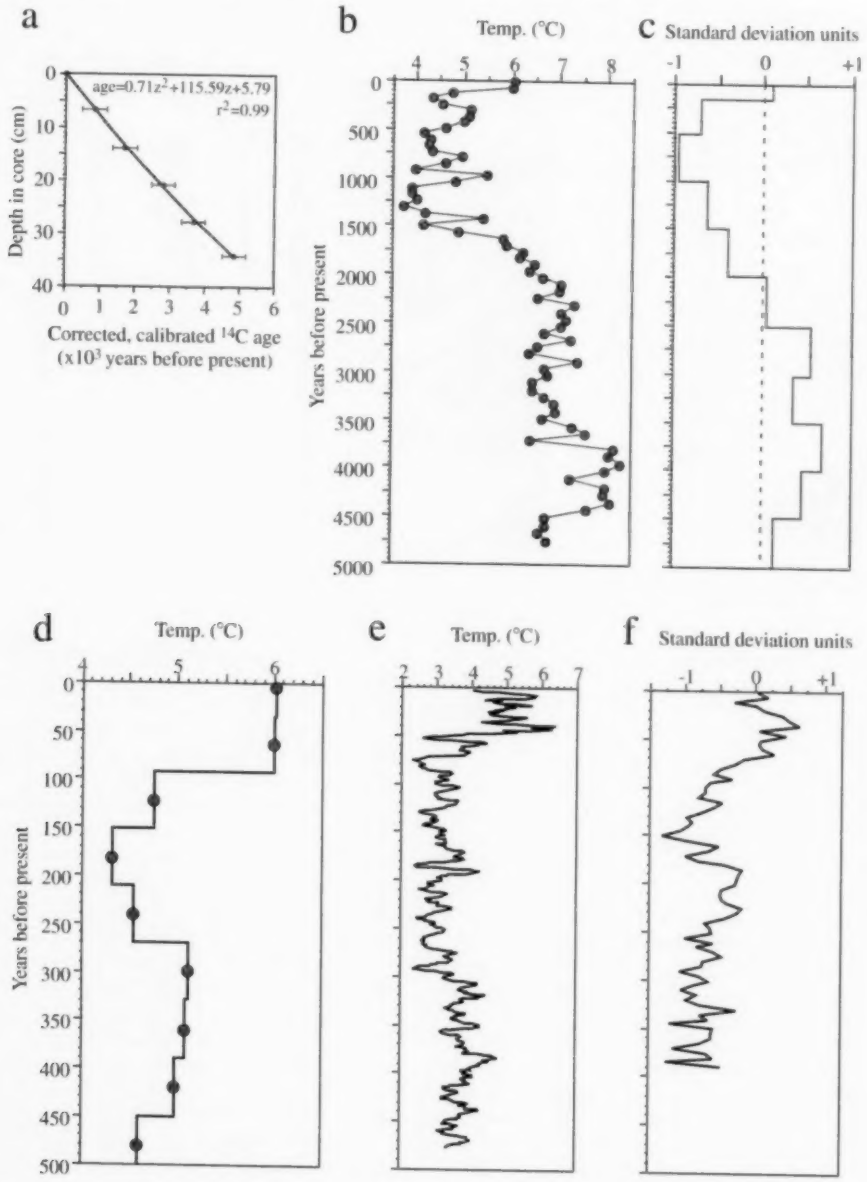
Fig. 10. Diatom-inferred summer lake water temperatures from the Fog Lake gravity core. The AMS ^{14}C age model in Fig. 10*a* has been used to convert depth to time in Figs. 10*b* and 10*d*. Water paleotemperature data are shown in relation to several independent regional paleoclimate proxies, including (*c*) pollen-derived summer temperature anomalies (Andrews et al. 1981), (*e*) spring air temperature from southern Baffin Island inferred from varve thicknesses (Hughen et al. 2000), and the (*f*) Overpeck et al. (1997) decadal record of circum-Arctic summer temperature anomalies.



selves (2.3–10.9°C), down-core reconstructions should be viewed with extreme caution in such cases.

Application to lake paleotemperature reconstruction

The summer surface water temperature inference model was applied to the 69 fossil assemblages from the Fog Lake gravity core, representing approximately 5000 years of continuous sediment deposition. The diatom stratigraphy is highly variable (Fig. 9), with alternating periods of dominance by *A. distans* and *S. exiguiformis*. The interval between 13.0 and 1.5 cm in the core preserves conspicuous increases in eunotioid taxa (*Eunotia vanheurkii*, *E. rhomboidea*, and *Peronia fibula*). Because these are strongly acidophilous taxa (Dixit et al. 1999), their increased representation suggests a marked lowering of lake water pH during this period of deposition. The upper 1.5 cm of the core also reveal rapid

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changes within the diatom flora: *Nitzschia perminuta*, which had previously been represented only in trace frequencies, increases to between 5 and 10% of assemblages, and *S. exiguiformis* also increases rapidly. Furthermore, *S. construens* var. *venter* disappears in the uppermost sediments. Although much of this detailed stratigraphic structure has only been revealed through contiguous, high-resolution analyses, it nonetheless remains problematic in terms of traditional qualitative interpretation, especially in terms of underlying paleoclimatic significance.

Reconstructed summer water temperatures have been plotted against time using the age model derived from AMS ^{14}C dating (Figs. 10*a* and 10*b*). Between 4800 and 2500 years before present, lake water temperature decreased gradually from a maximum of 8.2 to about 6.5°C. This reflects the lake temperature response to progressive regional cooling during the late Holocene, or Neoglaciation, as registered by multiple episodes of glacial advance on Baffin Island (Davis 1985). Accelerated lake water cooling ensued, with minimum reconstructed temperatures averaging 4°C attained between 1500 and 500 years before present. The initiation of this trend is also recorded by nearshore marine mollusc assemblages: faunas of arctic affinity migrated southward along the east coast of Baffin Island, eventually crossing the north coast of Cumberland Peninsula sometime after 3000 years before present (Dyke et al. 1996). These faunal migrations are associated with enhanced inputs of polar water masses emanating from the Queen Elizabeth Islands into Baffin Bay, possibly in response to a westward diversion of the Transpolar Drift. The effects of this oceanic reorganization are manifested in other terrestrial paleoenvironmental records, including the pollen record of Labrador, Keewatin, and Baffin Island (Andrews et al. 1981). Both the resemblance of the Fog Lake water temperature reconstruction to synthetic quantitative pollen inferences (Fig. 10*c*) and its coherence with Dyke et al.'s (1996) paleoceanographic scenario are striking.

Although the temporal resolution of the Fog Lake diatom record is somewhat compromised by extremely low sediment accumulation rates, the last 500 years are represented by the nine uppermost samples (Fig. 10*d*). Again, there is close correspondence between the Fog Lake water temperature reconstruction over this interval and the trends reconstructed at annual resolution from southern Baffin Island varve thicknesses (Fig. 10*e*) (Hughen et al. 2000) as well as those revealed by the decadal integration of multiple summer paleoclimate records, including both physically and biologically based proxies (Fig. 10*f*) (Overpeck et al. 1997). Summer water temperatures at Fog Lake appear to have increased by at least 1.5°C since the end of the Little Ice Age (Fig. 10*d*), which terminated the Neoglacial on Baffin Island. Although this warming trend was naturally initiated, at least some portion of twentieth century warming has been influenced by anthropogenic factors such as enhanced greenhouse gas emissions (Overpeck et al. 1997). The preliminary data suggest that the diatom record is fully capable of identifying such changes, whether causally related to natural variability or to human modification of the climate system.

In conclusion, a series of quantitative paleolimnological inference models have been developed from the modern diatom floras preserved in surface sediments from 61 Baffin Island lakes. These data complement similar efforts from the western Canadian Arctic (Pienitz et al. 1995) and northern Fennoscandia (Weckström et al. 1997; Rosén et al. 2000). However, this is the first training set developed for lakes situated entirely north of the tree line, thus extending the applicability of diatoms for paleotemperature reconstructions well into the Arctic tundra biome.

When applied down core, the summer water temperature inference model provides compelling similarities to independently constrained paleoclimate reconstructions, thereby attesting to the great promise of freshwater diatoms for paleoclimatic assessments in the eastern Canadian Arctic. The dense spatial distribution of lakes on Baffin Island implies an enormous potential for assembling regional quantitative climate reconstructions for this region based on fossil diatom stratigraphies. Furthermore, the fossil record of Baffin Island includes sites that escaped glacial modification during the late Pleistocene, so that the new inference models may be applied to sedimentary sequences as long as 90 000 years (Wolfe et al. 2000).

Although diatom communities may not necessarily express direct physiological responses to water temperature, climate clearly modulates indirect effects on the lake system that are registered by the diatom flora. Although the lakes considered in the present study are characterized by short algal growing seasons, intense lake ice regimes with regards to both thickness and duration, and low nutrient status, significant climatic and limnological controls over the composition of modern sediment diatom assemblages can be quantified using modern multivariate techniques. This result should stimulate further study of the rich fossil diatom record from Baffin Island, both at higher stratigraphic resolutions and over longer periods of recent earth history.

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Appendix. Locational and environmental data from the 61 study lakes used to constrain surface sediment diatom assemblages.

Lake	Lat. (°W)	Long. (°N)	Elev. (m asl)	Z_{max} (m)	Area (km^2)		Cond. ($\mu S \cdot cm^{-1}$)	Trans. (%)	pH	Summer water temperature (°C)
					Lake	Ws				
1	66.18	65.17	458.0	3.3	3.68	34.55	3.73	87.4	6.42	2.80
2	66.31	64.69	574.0	3.8	0.19	12.23	5.67	86.1	6.48	5.20
3	65.85	65.56	115.0	17.1	0.63	2.75	27.04	87.9	6.43	5.84
4	65.57	65.16	92.0	3.8	0.02	3.29	10.56	84.3	6.42	4.34
5	65.27	64.67	10.0	4.5	1.02	22.10	10.54	85.4	6.40	7.51
6	64.95	63.60	10.0	25.6	0.29	4.46	12.56	85.1	6.58	3.67
7	65.44	63.46	172.0	12.1	0.29	1.95	9.26	89.1	6.44	5.85
8	65.67	63.15	328.0	8.1	0.20	9.87	3.28	89.5	6.33	3.46
9	65.87	64.59	636.0	9.3	0.27	4.42	3.74	81.1	6.46	4.48
10	65.64	68.06	197.0	23.4	0.11	0.76	7.65	88.7	6.34	6.56
11	66.25	67.88	85.0	43.8	0.19	1.06	10.34	89.0	6.72	7.67
12	66.14	66.08	85.0	34.3	0.33	14.43	8.69	85.8	6.62	8.34
13	66.61	66.20	377.0	8.0	0.46	20.47	6.50	71.7	6.35	8.66
14	66.92	66.89	597.0	21.0	1.59	9.32	2.43	87.8	6.37	3.64
15	67.11	67.36	591.0	10.1	0.35	2.11	4.06	84.9	6.56	4.44
16	66.87	67.41	459.0	17.0	0.29	7.17	3.88	89.8	6.38	6.49
17	66.69	67.75	246.0	7.1	0.11	0.64	6.31	87.6	6.61	8.96
18	66.46	66.93	7.0	11.8	0.10	1.75	13.16	83.6	6.55	10.39
19	65.74	69.13	312.0	3.5	0.17	0.77	6.32	81.6	6.36	9.24
20	65.69	69.93	279.0	10.4	0.75	5.14	5.66	85.3	6.30	8.91
21	63.19	70.79	151.0	6.4	0.03	0.18	21.46	89.6	6.87	10.29
22	63.48	71.11	203.0	6.8	0.31	1.09	11.78	86.1	6.79	9.32
23	63.62	69.80	623.0	13.4	0.28	7.49	5.14	85.4	6.77	5.73
24	62.64	67.07	607.0	35.3	0.14	2.89	3.96	91.8	6.27	2.26
25	61.96	66.08	52.0	35.5	0.15	2.72	14.10	88.1	6.50	6.47
26	61.68	65.12	322.0	3.7	0.02	0.40	28.15	91.2	7.20	5.72
27	62.51	68.24	328.0	17.9	0.17	14.70	10.35	84.0	6.72	8.36
28	64.63	67.28	607.0	8.9	0.43	13.42	3.92	80.0	5.99	5.00
29	65.00	68.30	509.0	4.4	0.38	2.44	4.24	84.8	6.54	6.14
30	63.78	68.54	156.0	4.9	0.04	0.76	24.84	86.7	7.13	8.54
31	63.77	68.52	143.0	5.7	0.01	0.23	16.73	85.6	7.21	8.73
32	72.56	85.12	533.4	12.7	0.06	0.81	11.35	84.1	7.00	6.08
33	72.29	85.99	76.2	5.3	0.05	0.39	25.55	78.2	7.06	6.62
34	72.27	84.68	61.0	10.5	0.03	1.71	17.95	78.3	6.94	8.70
35	73.00	79.33	365.8	6.6	0.05	0.18	14.88	80.2	7.09	8.21
36	73.67	80.57	228.6	6.2	0.15	1.06	18.79	81.4	7.23	3.73
37	72.70	77.38	685.8	8.4	0.20	2.18	5.69	79.4	6.47	5.05
38	72.43	75.34	106.7	4.1	0.32	1.14	12.16	80.3	6.81	7.66
39	72.06	74.33	164.6	2.9	0.08	0.32	10.89	62.0	6.54	6.08
40	72.75	77.50	137.2	11.7	0.07	5.42	29.35	80.1	7.13	5.87
41	71.95	79.33	64.0	3.6	0.15	7.61	25.16	77.4	6.91	9.91
42	71.75	78.32	646.2	10.4	0.15	0.66	10.10	79.5	6.77	6.13
43	70.04	75.43	335.3	8.0	0.11	0.64	10.06	77.6	6.59	9.23
44	70.27	76.06	152.4	6.2	0.06	1.17	11.29	71.1	6.69	8.60
45	70.74	74.79	438.9	5.3	0.01	0.03	14.42	75.2	6.87	8.28
46	70.48	70.66	91.4	10.2	0.06	1.15	14.49	80.9	6.50	8.26
47	70.54	72.52	542.5	7.9	0.13	0.47	9.34	77.9	7.17	7.88
48	70.96	70.86	134.1	13.2	0.09	4.66	10.02	83.5	6.19	7.88
49	70.46	68.71	109.7	8.1	0.20	0.75	16.77	72.1	7.25	6.40
50	69.89	67.82	158.5	13.6	0.14	0.80	13.35	81.7	6.42	6.28
51	69.42	67.31	207.3	21.0	0.08	0.78	9.15	78.5	5.96	3.47
52	69.76	70.65	579.1	14.6	0.33	4.39	17.80	78.7	7.08	7.17
53	69.88	70.41	112.8	4.8	0.09	5.57	25.08	76.5	7.36	10.94
54	70.29	69.33	295.7	20.4	0.28	2.25	14.91	85.2	6.48	7.40
55	63.60	65.52	597.4	17.9	0.65	6.94	0.96	87.8	7.11	2.75
56	67.80	66.28	79.2	6.5	0.16	8.31	6.41	76.7	6.68	7.91

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Air temperature (°C)			Precipitation (mm)		
January	July	Annual	January	July	Annual
–26.3	6.5	–10.3	19	45	392
–25.3	6.6	–10.4	18	39	376
–26.1	6.7	–9.8	16	45	358
–25.6	6.8	–9.8	18	49	385
–24.4	6.7	–9.9	19	50	412
–23.6	7.3	–9.7	24	56	497
–24.2	7.6	–9.9	23	47	435
–23.2	6.6	–9.3	25	49	522
–26.0	6.6	–10.2	20	45	399
–27.0	7.1	–10.1	11	44	305
–26.7	6.8	–10.5	10	42	305
–26.4	6.4	–10.3	14	38	336
–26.8	6.1	–10.8	16	42	366
–27.1	6.0	–11.0	11	36	337
–27.3	5.8	–11.1	12	40	343
–27.0	6.1	–11.0	10	35	318
–26.5	6.5	–10.8	10	38	317
–25.7	6.7	–10.2	13	38	326
–27.0	7.2	–10.3	10	45	289
–27.0	7.4	–10.4	10	43	270
–24.1	7.6	–8.9	13	47	314
–23.5	7.7	–9.2	13	48	311
–25.4	6.9	–9.6	14	49	315
–24.2	5.5	–8.5	23	59	393
–22.5	5.8	–7.3	23	54	374
–21.5	5.3	–6.9	29	56	415
–23.9	6.5	–8.4	18	52	348
–26.7	6.6	–10.0	17	56	360
–27.1	7.0	–10.1	13	51	318
–24.8	6.8	–9.5	13	48	330
–24.8	6.8	–9.4	13	48	330
–31.4	5.5	–15.5	3	25	162
–31.3	5.8	–15.1	4	24	162
–30.9	5.8	–14.7	3	27	164
–30.3	5.6	–14.6	6	27	159
–29.6	5.2	–14.2	5	26	157
–30.7	5.0	–14.1	6	26	193
–30.5	5.5	–13.2	7	25	197
–29.9	6.1	–12.4	7	22	208
–30.6	5.1	–14.1	6	26	190
–30.7	5.8	–14.0	5	27	178
–31.4	5.8	–14.4	5	26	196
–30.1	6.7	–13.6	5	28	196
–30.3	6.6	–13.6	6	29	193
–30.8	6.0	–13.5	7	30	196
–29.1	5.0	–12.5	8	25	202
–29.8	5.6	–13.2	8	29	206
–29.1	5.0	–12.2	7	21	207
–27.6	4.9	–12.2	9	23	191
–27.5	5.8	–10.8	10	24	195
–27.2	5.7	–11.5	9	21	193
–29.1	5.5	–12.9	8	31	223
–28.9	5.5	–12.6	9	31	220
–28.2	5.3	–11.4	9	26	197
–25.3	5.0	–9.4	27	63	456
–26.7	5.4	–11.5	11	28	284

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Table A1 (*concluded*).

Lake	Lat. (°W)	Long. (°N)	Elev. (m asl)	Z_{max} (m)	Area (km^2)		Cond. ($\mu S \cdot cm^{-1}$)	Trans. (%)	pH	Summer water temperature (°C)
					Lake	Ws				
57	67.70	65.34	438.9	55.7	0.46	8.93	1.46	86.9	6.24	5.21
58	66.80	63.96	18.3	11.0	0.20	1.62	30.11	85.4	4.66	9.84
59	66.95	62.90	201.2	14.7	0.18	2.90	1.21	75.4	6.03	5.20
60	66.91	62.17	152.4	18.2	0.08	1.50	7.45	80.4	5.35	4.24
61	67.34	63.61	371.9	48.9	0.11	0.50	4.25	87.4	6.04	4.45
Maximum			685.8	55.7	3.7	34.6	30.1	91.8	7.4	10.9
Minimum			7.0	2.9	0.01	0.03	1.21	62.0	4.7	2.3
Range			678.8	52.8	3.6	34.2	28.9	29.8	2.7	8.6
Mean			285.5	13.3	0.3	4.6	11.5	82.7	6.6	6.7
SD			205.6	11.4	0.5	6.3	7.7	5.7	0.5	2.1

Note: Lat., latitude; Long., longitude; Elev., lake elevation; Z_{max}, maximum lake depth; Ws, watershed; Cond, conductivity; Trans., transmissivity (25-cm path at 660 nm).

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Air temperature (°C)			Precipitation (mm)		
January	July	Annual	January	July	Annual
–26.4	5.0	–11.5	13	28	283
–25.0	5.7	–10.1	22	42	399
–23.0	7.1	–8.2	21	31	392
–23.3	6.3	–9.6	25	35	453
–25.4	5.3	–10.8	15	27	305
–21.5	7.7	–6.9	29	63	522
–31.4	4.9	–15.5	3	21	157
9.9	2.8	8.6	26	42	365
–27	6.1	–11.2	13	37	296
2.6	0.8	2.0	7	11	96

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Testing assumptions of otolith radiometric aging with two long-lived fishes from the northern Gulf of Mexico

M. Scott Baker, Jr., Charles A. Wilson, and Daniel L. VanGent

Abstract: In this study, we evaluate the assumptions for estimating ages with radiometric and elemental analyses of otoliths of red snapper (*Lutjanus campechanus*) and red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico. In whole otoliths of both red snapper and red drum, ^{226}Ra activity was inversely correlated with the otolith mass increase with age, validating the assumption that ^{226}Ra accumulation in otoliths occurs at a rate proportional to that of Ca. ^{222}Rn emanation from otoliths decreased as otolith mass increased. ^{222}Rn loss occurred in all red snapper otolith samples (≤4.1%) and, to a lesser extent, in red drum otolith samples (≤0.6%) and decreased with increasing size (age) of the otolith. For red drum, the assumption that the initial activity ratio of ^{210}Po to ^{226}Ra in otoliths is essentially zero was indirectly validated with elemental analyses of Pb and Ba. Radiometric age estimation of shallow-water fishes from the northern Gulf of Mexico holds great promise, considering that ^{226}Ra activities observed in this study are some of the highest recorded to date for species to which the ^{210}Pb/^{226}Ra dating technique has been applied.

Résumé : On trouvera ici une évaluation des présuppositions de la méthode d'estimation de l'âge par radiométrie et analyse des éléments dans les otolithes du Vivaneau rouge, *Lutjanus campechanus*, et du Tambour rouge, *Sciaenops ocellatus*, du nord du golfe du Mexique. Dans les otolithes entiers des deux espèces, l'activité du ^{226}Ra est en corrélation inverse avec croissance de la masse de l'otolithe avec l'âge, ce qui valide la présupposition qui veut que le taux d'accumulation du ^{226}Ra soit proportionnel à celui du calcium. L'émanation du radon-222 décroît avec l'augmentation de la masse de l'otolithe. Il y a une perte de radon-222 dans tous les échantillons d'otolithes de Vivaneaux rouges (≤4,1%) et, à un degré moindre, dans ceux des Tambours rouges (≤0,6%); la perte diminue cependant avec l'augmentation de taille (âge) de l'otolithe. Chez le Tambour rouge, la présupposition que l'activité initiale du rapport ^{210}Po/^{226}Ra est essentiellement nulle se vérifie indirectement par les analyses de Pb et de Ba. L'estimation de l'âge par radiométrie chez les poissons d'eau peu profonde du nord du golfe du Mexique semble être une méthode très prometteuse; en effet, les niveaux d'activité du ^{226}Ra observés ici sont parmi les plus élevés obtenus chez des espèces soumises à la technique d'estimation de l'âge au moyen du rapport ^{210}Po/^{226}Ra.

[Traduit par la Rédaction]

Introduction

Radiometric analysis of naturally occurring ^{226}Ra and its daughter elements in otoliths has been used to validate age estimates derived from visible otolith annuli in a number of fish species (Bennett et al. 1982; Campana et al. 1990; Andrews et al. 1999*a*). In most studies, radiometric validation of otolith annulus age estimates proved successful. Despite these successes, few studies have addressed all of the assumptions for valid radiometric analysis. These assumptions were first detailed by Faure (1986) and later modified by Bennett et al. (1982).

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Radiometric aging of fish otoliths relies on the principle assumptions that (1) ^{226}Ra is naturally incorporated into the otolith either at a constant rate or at a rate that is proportional to the mass increase of the otolith, (2) the otolith remains a chemically closed system and there are no losses or gains of radionuclides (e.g., ^{222}Rn), and (3) the initial activity ratio of ^{210}Pb to ^{226}Ra is close to zero. In addition to these assumptions, the general flux of ^{226}Ra within the environmental system to be studied (i.e., Gulf of Mexico) should be known or at least understood.

Assumption 1 has been generally ignored in previous studies if the first year or years of growth (cores) were isolated from the whole otolith or if averaged measurements of ^{226}Ra were used for each pooled otolith sample (Campana et al. 1990; Milton et al. 1995; Andrews et al. 1999*a*). Regardless of whether whole otoliths or otolith cores are used, the dynamics of ^{226}Ra uptake need to be understood for the species in question.

Assumption 2, concerning ^{222}Rn loss, is in need of further examination (Fenton and Short 1992; West and Gauldie 1994; Kimura and Kastelle 1995). It is possible that ^{222}Rn could be lost from within the otolith due to the characteristics of the gas and the generalized internal structure of the otolith (West and Gauldie 1994). Two previous studies have

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tried to test for ^{222}Rn degassing from fish otoliths. Whitehead and Ditchburn (1995) used alpha spectrometry to assess ^{222}Rn loss from orange roughy (*Hoplostethus atlanticus*) otoliths and concluded that mean ^{222}Rn loss was negligible. Gauldie and Cremer (1998) applied gamma spectrometry to the same species and reported that detectable quantities of ^{222}Rn diffused out of orange roughy otoliths, resulting in a wide range of estimated loss (below detection to 8.9%). They stated that corrections for ^{222}Rn loss would be uncertain given the variability in loss of ^{222}Rn among otoliths of different sizes. One problem with the aforementioned studies is that the ^{226}Ra activity (and thus potential ^{222}Rn loss) in orange roughy otoliths is very low and the resulting ^{222}Rn activities are close to instrument detection limits. Advanced technology now allows for measurement of ^{222}Rn in water with a photon–electron rejecting alpha liquid scintillation (PERALS) spectrometer. The PERALS method differs from conventional radiometric dating techniques in that it requires much smaller samples (≥100 mg), has comparable alpha energy resolution, and can separate alpha particles from beta/gamma backgrounds by a simple one-knob pulse shape discriminator adjustment. The alpha counting efficiency is reputed to be a constant 99.7% with backgrounds as low as 0.001 disintegrations per minute (dpm) in the 4–7 MeV range, thus allowing for much shorter counting times (Case and McDowell 1990). In addition, a standard (with known ^{226}Ra activity and ^{222}Rn emanation characteristics) should be used as a guideline for an otolith emanation study. Barite ($BaSO_4$) has a crystalline structure similar to $CaCO_3$ and has been observed to leak ^{222}Rn at a rate of approximately 3% (D.L. VanGent, unpublished data). Significant loss of ^{222}Rn from otoliths, which eventually leads to a lack of measurable ^{210}Pb, would result in an underestimation of age.

Assumption 3 concerns the initial activity ratio in samples and is untested in most radiometric studies. The real concern, however, is allogenic (coming from outside the otolith) ^{210}Pb and its granddaughter ^{210}Po. Because ^{210}Pb is a beta emitter and difficult to analyze, the α-emitting granddaughter ^{210}Po is used as its proxy when samples are sufficiently old (four to five half-lives or ≥2 years) such that the two radionuclides are in secular equilibrium with each other. Fishes can accumulate ^{210}Po through the food chain (Heyraud and Cherry 1979), and it is possible that ^{210}Po may be incorporated into the otolith (Grady et al. 1989). Therefore, allogenic ^{210}Po could be present in otoliths in addition to that which is present from the natural decay of ^{226}Ra. Because ^{210}Po has such a short half-life, waiting for 2 years after sample collection should alleviate this problem (Andrews et al. 1999*a*). Investigators have assumed that uptake of allogenic ^{210}Pb and ^{210}Po is greatly exceeded by the uptake of ^{226}Ra, and thus, allogenic ^{210}Pb and ^{210}Po can be ignored in subsequent calculations (Fenton et al. 1990; West and Gauldie 1994). The initial activity ratio has been tested both by measuring Pb and Ba as stable proxies of ^{210}Pb and ^{226}Ra and through radiometric analysis of the youngest available age groups (Milton et al. 1995; Stewart et al. 1995; Andrews et al. 1999*a*). Juvenile fishes are best suited for testing this assumption because they are not old enough to have a significant amount of ^{210}Pb from ingrowth from ^{226}Ra; therefore, any ^{210}Po present in the otolith would be from exogenous sources. Several researchers in past studies assumed the value of the initial activity ratio to be zero simply because the necessary age category of fishes was not available for radiometric dating (Bennett et al. 1982; Campana et al. 1990; Fenton and Short 1995). Any allogenic ^{210}Po present in otoliths and not accounted for would increase radiometric age estimates.

This study was designed to test and evaluate the three assumptions necessary for valid radiometric aging with otoliths of red snapper (*Lutjanus campechanus*) and red drum (*Sciaenops ocellatus*) from the northern Gulf of Mexico. These species were selected because they are of great economic importance and because ages determined from otolith sections have been validated to at least 30 years in red snapper (Wilson and Nieland 2001) and 27 years in red drum (Murphy and Taylor 1991). Our specific objectives were to (*i*) determine and compare ^{226}Ra activity in whole otoliths of different age groups, (*ii*) quantify any ^{222}Rn loss from whole otoliths, (*iii*) determine if stable Pb and Ba provide the equivalent of ^{210}Pb to ^{226}Ra in the youngest otolith samples, and (*iv*) discuss what impacts the Mississippi River may have on ^{210}Pb/^{226}Ra dating of fish otoliths from the northern Gulf of Mexico.

Materials and methods

Otolith collection and section preparation

The red snapper and red drum otoliths used in this study were drawn from the archive collection of the Louisiana State University Coastal Fisheries Institute held in Baton Rouge, Louisiana. These collections were obtained from the northern Gulf of Mexico commercial and recreational harvests of 1986 through 1998. All red snapper and red drum otoliths used in this study were prepared, sectioned, and aged following the procedures described in Render (1995) and Beckman et al. (1988), respectively.

^{226}Ra in otoliths

Because Ra is a Ca analogue, the rate of accumulation of ^{226}Ra in otoliths through time is assumed to occur at a rate that is in constant proportion to that of Ca. In order to test this assumption, otoliths segregated by species and age group were nondestructively analyzed with a high-purity intrinsic germanium gamma detector (HPGe). Whole sagittae were pooled together to obtain the minimum sample weight of 70 g necessary for HPGe analysis. The closed-ended coaxial detector has a 98-cm^3 active volume and is 5.5 cm in diameter and 4.4 cm in length. The detector was coupled with an 8192 multiple-channel analyzer (Ortec buffer card). The resolution is 2.1 keV at 1332 keV. All samples were subjected to the energy and efficiency calibration of a NIST traceable mixed gamma standard (serial M27304 from Amersham Corp.) enclosed in a 100 × 15 mm petri dish.

The petri dish geometry was chosen for the otolith ^{226}Ra measurements because of the "thin" sample configuration. The typical linear thickness of an otolith sample of any size with group mass of 70 g was 0.5 cm. This obviated for all practical purposes problems with sample self-absorption. In order to verify this experimentally, $CaCO_3$ chips with a known content of ^{226}Ra (9.9 ± 1.0 dpm·g^{-1}) of various sizes approximating the size range of fish otoliths and weighing 70 g were run for 100 000 s each on the HPGe system in the petri dish geometry. The greatest ^{226}Ra content variation among sample sizes was found to be <5%.

The sample containers were put into a plastic bag and placed directly on the spectrometer end cap inside an Ortec ultralow background shield (model GC2521). All gamma spectra were allowed to collect for up to 300 000 s. The ^{226}Ra activity was directly mea-

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sured from its 186-keV gamma ray peak. We assumed no interference from the 143-keV gamma ray peak (^{235}U) in all otolith samples because no activity was ever recorded in this region. A 300 000-s background (bare detector) count was "manually" stripped from each spectrum prior to analysis in order to account for energy shifts that may have occurred sample to sample. The relationship between ^{226}Ra and mean otolith weight and ^{226}Ra and mean otolith age was plotted for both red snapper and red drum.

^{222}Rn emanation and loss

Measurement of ^{222}Rn in water was conducted with a PERALS spectrometer. Alpha measurements were quantified with a Dell 450/L personal computer fitted with a multichannel buffer and Maestro software (model A65-B1, version 3.06; Ortec, Inc.). Otoliths used in the ^{222}Rn emanation analyses were sorted by species and otolith weight. Pooled groups of otoliths approximately 10 g each were placed into individual 20-mL borosilicate glass vials. The vials were filled to capacity with double-distilled deionized (DDI) water so that no air space was left when the polyseal cap was attached. The DDI water used in this study was produced onsite and typical blanks were (via radon Bubbler and Lucas cell EPA/600/2–87/082) approximately 0.002 dpm ^{222}Rn·mL^{-1}. A standard containing 111 dpm ^{226}Ra and a reagent blank containing only water were created exactly as the samples. To allow for the ingrowth of ^{222}Rn, ^{218}Po, and ^{214}Po from the ambient ^{226}Ra within each otolith sample, all vials were stored away from light and excessive heat in a refrigerator. After a period of 3 weeks, ^{222}Rn (half-life 3.82 days) and all of its daughters were assumed to be in secular equilibrium (Cochran 1982).

The Rn-in-water sampling procedure used in this study was adapted from the procedure developed by McDowell (1999). The procedure requires use of a 50-mL graduated hypodermic glass syringe with a valve attached to the lip. In addition, two specialty needles were used: a 22-gauge 7.62-cm stainless steel straight needle and a 22-gauge 15.24-cm 160° stainless steel curved needle. With the syringe and a straight 7.62-cm needle, 3 mL of POLEX, a proprietary extractive scintillator available from East Tennessee Radiometric/Analytical Chemicals, Inc. (ETRAC), was drawn into the syringe and the valve closed. At the time of sample extraction, the valve was opened and approximately 12.5 mL of water was drawn in from the sample vial. In order to maximize recovery, the water to organic ratio should not be greater than 10 to 1 (Hamanaka et al. 1998). After the water was drawn into the syringe, the needle was removed, the valve closed, and the syringe agitated for 5–10 min with sufficient vigor to disperse the organic scintillator into the aqueous phase. The syringe was then placed in an inverted position to allow for phase separation to occur. The curved needle was then attached to the syringe, the valve opened, and two or three drops of the organic phase was flushed out the valve and needle. Prior to sample injection, a 1-mL radon liquid scintillation counting cell, supplied by ETRAC, was flushed with dry argon or nitrogen with a straight 22-gauge 15.24-cm needle as a sparging lance. One millilitre of the organic phase was injected into the cell and the opening sealed with silicone gel. Prior to counting on the PERALS spectrometer, sealed samples were left undisturbed for approximately 4 h to allow secular equilibrium to be established between Rn and the two Po daughters.

Samples, blanks, and standards were counted on the PERALS spectrometer for approximately 3 days. Recovery of a 111 dpm ^{226}Ra standard (50.28 %) was used to estimate the recovery of the ^{226}Ra daughters in each emanation sample and the blank. In addition to the Ra in water standard, 3 g of barite with known ^{226}Ra activity was prepared following the aforementioned guidelines and the percent ^{222}Rn loss was compared with that observed in otoliths. The sample blank (containing only DDI water) was 0.0787 dpm for all energy regions occupied by the ^{222}Rn, ^{218}Po, and ^{214}Po peaks combined. The pulse shape discriminator setting for all samples was derived by first analyzing the 111 dpm ^{226}Ra standard in pulse shape mode on the PERALS spectrometer.

^{222}Rn loss (expressed as a percentage with a maximum of 100) was calculated with the formula

$$^{222}\text{Rn loss}_A = \frac{^{222}\text{Rn}_A \text{ activity (dpm·g}^{-1} \pm 1 \text{ SD)}}{^{226}\text{Ra}_A \text{ activity (dpm·g}^{-1} \pm 1 \text{ SD)}}.$$

Initial activity ratio

The initial activity ratio of ^{210}Pb to ^{226}Ra was estimated by determining the ratio of stable Pb to Ba in juvenile red drum (n = 74) otoliths collected from the northern Gulf of Mexico in 1998. Elemental analyses were performed using inductively coupled plasma mass spectrometry. Concentrations of Pb, Ba, and the Pb/Ba ratio were plotted against otolith weight. Young-of-the-year red snapper were not available for this study; however, Ba concentrations from Patterson et al. (1998) (Pb was not analyzed) were compared with Ba concentrations found in red drum and other estuarine fishes. Procedures describing otolith sample processing and elemental analyses were similar to those described elsewhere (Patterson et al. 1998).

Results

^{226}Ra activity in otoliths

Prior to radiometric analysis of whole otoliths, 5948 records (3001 red snapper and 2947 red drum) were used to generate an otolith weight to age relationship for each species (Fig. 1). A general linear test (with ln-transformed data) indicated that separate models were needed for each species (analysis of covariance (ANCOVA) test for homogeneity of slopes, df = 1, MS = 61.316, F = 966.24, P = 0.0001; ANCOVA test for equal intercepts, df = 1, MS = 175.55, F = 2766, P = 0.0001). The resulting equations were calculated for each species:

$$\text{Red snapper: OW (g)} = 1.2906 \times \text{age (years)} - 2.6766 \ (r^2 = 0.68)$$

$$\text{Red drum: OW (g)} = 0.7356 \times \text{age (years)} - 1.0519 \ (r^2 = 0.93)$$

where OW (g) is ln(otolith weight) and age (years) is ln(otolith section age).

^{226}Ra activity of whole otoliths was highest in young fishes and gradually decreased in older fishes (Table 1). ^{226}Ra specific activities versus mean otolith weight and mean otolith section age for red snapper and red drum are plotted in Fig. 2. General linear tests indicated that separate models were needed for each species for otolith weight (ANCOVA test for homogeneity of slopes, df = 1, MS = 0.0059, F = 0.05, P = 0.8339; ANCOVA test for equal intercepts, df = 1, MS = 2.579, F = 22.06, P = 0.0093) as well as age (ANCOVA test for homogeneity of slopes, df = 1, MS = 0.0567, F = 0.60, P = 0.4805; ANCOVA test for equal intercepts, df = 1, MS = 1.521, F = 16.22, P = 0.0158). The resulting equations were calculated for each species:

$$\text{Red snapper: } ^{226}\text{Ra} = -0.5698(\ln \text{otolith weight (g)}) - 2.1144 \ (r^2 = 0.77)$$

$$^{226}\text{Ra} = -0.6396(\ln \text{age (years)}) - 0.9154 \ (r^2 = 0.77)$$

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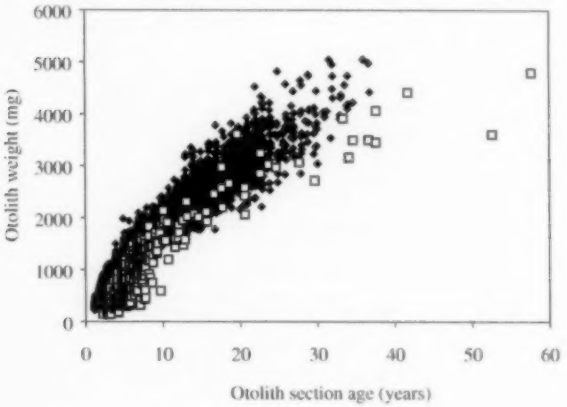
Fig. 1. Relationship between otolith weight and otolith section age for red snapper (□) (n = 3001) and red drum (■) (n = 2947) from the northern Gulf of Mexico.



$$\text{Red drum: } {}^{226}\text{Ra} = -0.6411(\ln \text{otolith weight (g)}) - 0.9388 \quad (r^2 = 0.79)$$

$${}^{226}\text{Ra} = -0.4414(\ln \text{age (years)}) - 0.3791 \quad (r^2 = 0.88).$$

Counting errors for predicted ^{226}Ra activities were assumed to be 14% for red snapper and 8% for red drum, as these were the mean counting errors observed in actual samples. ^{226}Ra activities for each 10-g emanation sample were not measured with the gamma detector used in this study because of instrument configuration restrictions. Therefore, ^{226}Ra activities used to calculate ^{222}Rn loss from pooled red snapper and red drum otoliths was estimated using the ln-transformed relationship between ^{226}Ra activity and mean otolith weight shown above.

^{222}Rn emanation and loss

^{222}Rn emanation from otoliths was detected but appeared to decrease with increasing size (age) of the otolith (Fig. 3). ^{222}Rn emanation rate was not affected by otolith weight or species (ANCOVA test for homogeneity of slopes, df = 1, MS = 4.6×10^{-6}, F = 0.29, P = 0.6298; ANCOVA test for equal intercepts, df = 1, MS = 1.62×10^{-5}, F = 1.00, P = 0.3900). Positive ^{222}Rn emanation was present in all otolith samples examined except for one red drum sample that did not register counts above background (Table 2). The ^{222}Rn emanation rate from red snapper otoliths ranged from 0.0154 ± 0.0025 dpm·g^{-1} ± 1 SD in the 1-year-old sample to 0.0022 ± 0.0002 dpm·g^{-1} ± 1 SD in the 5-year-old sample. The ^{222}Rn emanation rate from red drum otoliths ranged from 0.0073 ± 0.0008 dpm·g^{-1} ± 1 SD in the 0.7-year-old sample to below background in the 1.8-year-old sample.

As with ^{222}Rn emanation values, ^{222}Rn loss appeared to decrease with increasing mean size (age) of the otolith (Fig. 3). Rate of ^{222}Rn loss was not affected by otolith weight but was significantly different between species (ANCOVA test for homogeneity of slopes, df = 1, MS = 0.5196, F = 1.10, P = 0.3707; ANCOVA test for equal intercepts, df = 1, MS = 7.509, F = 15.94, P = 0.0281). Based on the above ^{222}Rn emanation findings and predicted ^{226}Ra age-specific activities, ^{222}Rn loss from within otoliths occurred in all but one pooled otolith sample (Table 2). ^{222}Rn loss from red snapper otoliths ranged from 4.1 ± 0.9% in the smallest otoliths (mean age = 1 year) to 1.6 ± 0.3% in the largest otoliths (mean age = 5 years). ^{222}Rn loss from red drum otoliths ranged from 0.6 ± 0.1% in the smallest otoliths (mean age = 0.7 year) to below background levels in the largest otoliths (mean age = 1.8 years).

Initial activity ratio

The red drum juveniles used for stable elemental analyses were collected in 1998 and processed in 1999. These fish ranged in age from 0.5 to 3 years, with the majority being approximately 1 year old (thus making their calendar birth date between 1997 and 1995). Therefore, the majority of samples were processed approximately 2 years after their birth date, and for these samples, we assumed that ^{210}Pb was in secular equilibrium with ^{210}Po. Mean Pb and Ba concentrations (parts per million (ppm) ± SD) in 74 juvenile red drum were 0.0067 ± 0.0045 and 21.86 ± 13.83, respectively, resulting in a Pb/Ba ratio of 0.0005 ± 0.0005. This Pb/Ba ratio, if acting as a stable proxy for ^{210}Pb/^{226}Ra, would indicate a radiometric age of 0.016 ± 0.016 year with the equation

$$t = \frac{-1}{\lambda_p}\left[\frac{\ln(1-S)}{1-R_0}\right]$$

where t is age (years), λ_P is the decay constant for ^{210}Pb (0.031·year^{-1}), $S = (^{210}\text{Pb}/^{226}\text{Ra})_t$ is the activity at time t assuming insignificant ^{222}Rn loss, and $R_0 = (^{210}\text{Pb}/^{226}\text{Ra})_t$ is the initial activity ratio at t = 0. Only Ba was significantly negatively correlated with otolith weight (r = –0.26, P = 0.0254, n = 74) (Fig. 4). This correlation is undoubtedly due to the significant differences in Ba concentrations by location (F = 39.54, P = 0.001). All red drum otoliths ≥0.6 g were taken from Tampa Bay, Florida, a water body shown to have low levels of Ba in relation to the other northern Gulf of Mexico estuaries where red drum were collected for this study.

Discussion

We attempted to address all of the assumptions necessary for otolith radiometric dating of red snapper and red drum from the northern Gulf of Mexico and provided quantitative estimates of ^{226}Ra activity and ^{222}Rn leakage for both species. ^{226}Ra activities in the otoliths analyzed in this study were some of the highest reported in the literature and to which the ^{210}Pb/^{226}Ra disequilibria dating technique has been applied.

The assumption that the otolith remains a chemically closed system in terms of ^{222}Rn loss from within the otolith appears to be false. ^{222}Rn loss was present in all red snapper samples and in all but the largest (mean otolith size) of the red drum samples. In the two species observed here, the maximum loss of ^{222}Rn (≤4.1%) from whole otoliths is probably insufficient to significantly affect radiometric age estimates based on the errors associated with the ^{210}Pb/^{226}Ra dating techniques currently used. Nevertheless, this study demonstrated both that ^{222}Rn emanation from otoliths was present and that the assumption that the otolith is a chemi-

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Table 1. Results of ^{226}Ra activity analyses of whole sagitta from red snapper and red drum from the northern Gulf of Mexico.

Species	No. of otoliths in sample	Sample weight (g)	Mean otolith weight (g ± 1 SD)	Mean otolith section age (years ± 1 SD)	^{226}Ra activity (dpm·g^{-1} ± 1 SD)
Red snapper	257	71.11	0.279±0.074	2.20±0.52	0.3125±0.0720
	123	72.70	0.601±0.314	4.08±1.65	0.1075±0.0108
	48	72.45	1.510±0.281	8.09±2.64	0.1135±0.0137
	34	72.97	2.207±1.061	14.79±5.87	0.0776±0.0092
Red drum	183	71.15	0.383±0.203	0.87±0.58	0.6510±0.0402
	52	70.97	1.370±0.217	4.38±1.29	0.4718±0.0321
	31	70.10	2.208±0.749	12.81±6.85	0.1851±0.0185
	30	70.30	2.234±0.229	12.97±3.12	0.2239±0.0201

Fig. 2. ^{226}Ra activity (±SD) plotted against (*a*) mean otolith weight (±SD) and (*b*) mean otolith age (±SD) for red snapper (▲) and red drum (■) from the northern Gulf of Mexico. Each data point represents one 70-g sample except for the largest (oldest) red drum sample (n = 2). ^{226}Ra data for blue grenadier (●) (from Fenton et al. 1990) have been added for comparative purposes only. Data points for each species were fitted with a trend line to illustrate the ^{226}Ra uptake pattern.

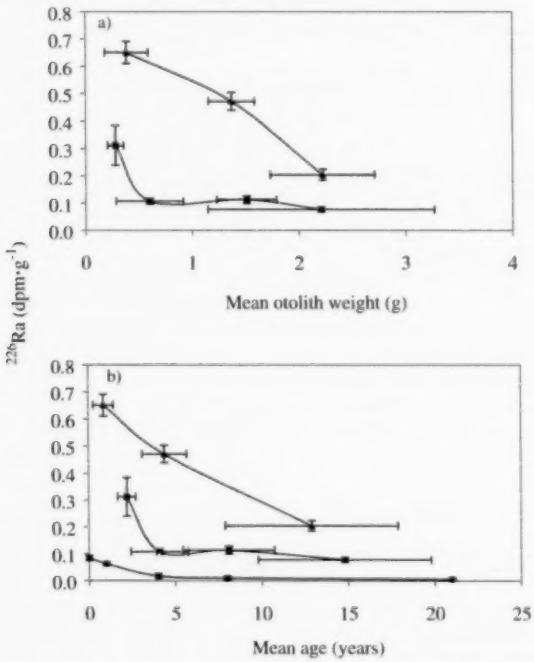
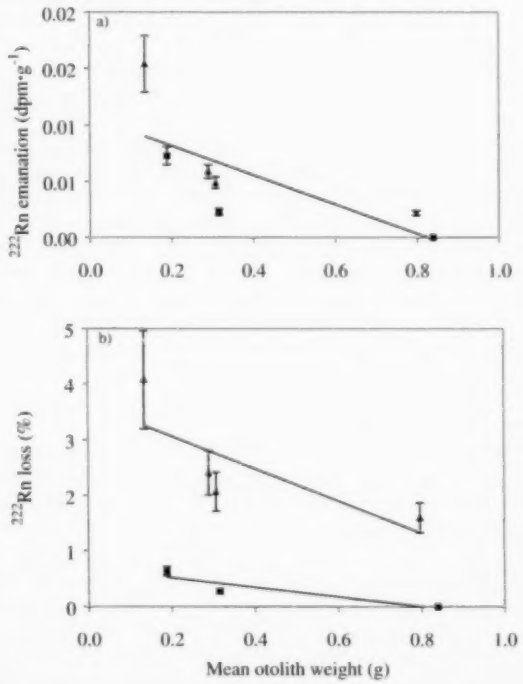


Fig. 3. (*a*) ^{222}Rn emanation (±SD) and (*b*) ^{222}Rn loss (±SD) plotted against mean otolith weight (±SD) for red snapper (▲) (n = 4) and red drum (■) (n = 3) pooled otolith samples from the northern Gulf of Mexico. ^{222}Rn emanation rates by otolith weight for red snapper and red drum were determined to be not statistically different between species. ^{222}Rn loss was determined to be statistically different for each species and was therefore fitted with linear trend lines for comparative purposes.



cally closed system was compromised at least for red snapper and red drum.

Based on previous studies concerning ^{222}Rn degassing from otoliths and the structural characteristics of $CaCO_3$, Rn loss is thought to be minimal (Campana 1999). A wide range of Rn emanation rates and Rn loss estimates exist for various matrices. Values of ^{222}Rn loss from various rock samples range from below detection to 18% (Rama and Moore 1984; Krishnaswami and Seidemann 1988). Lyons et al. (1989) observed practically no net loss of ^{222}Rn from speleothem $CaCO_3$. ^{222}Rn loss from barite, an elemental compound with a structure similar to that of $CaCO_3$, was shown to be 3.3 ± 0.6% in this study. ^{222}Rn degassing from large and small orange roughy otoliths was calculated to be 0.6 ± 0.6 and 1.5 ± 0.6%, respectively (Whitehead and Ditchburn 1995). For the same species, Gauldie and Cremer (1998) found ^{222}Rn loss to range from 0 to 8.9%. In this study, ^{222}Rn degassing from red snapper and red drum otoliths appeared to decrease with increasing mean size (age) of the otolith.

The data provided by this study indicated that ^{222}Rn emanation was dependent on factors such as the shape, size, and surface area of the otolith. Lutjanid otoliths, like those of red snapper, are generally thinner and more concave than those of sciaenids and as a result have more surface area. As a

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Table 2. Results of ^{222}Rn emanation analyses for red snapper (RS) and red drum (RD) otoliths.

Sample ID	Total weight (g)	Mean otolith weight (g)	Mean age (years)	^{222}Rn emanation (dpm·g^{-1} ± 1 SD)	^{226}Ra activity (dpm·g^{-1} ± 1 SD)	^{222}Rn loss (% ± 1 SD)
Ba-std	3.10	—	—	28.90±5.38	888±44	3.25±0.63
RS-1	1.89	0.135	1	0.0154±0.0025	0.3778±0.0529	4.08±0.88
RS-6	10.14	0.289	3	0.0059±0.0006	0.2449±0.0343	2.41±0.40
RS-8	10.20	0.307	3	0.0049±0.0005	0.2366±0.0331	2.07±0.35
RS-12	10.37	0.797	5	0.0022±0.0002	0.1374±0.0192	1.60±0.27
RD-1	10.13	0.188	0.7	0.0073±0.0008	1.1400±0.0912	0.64±0.08
RD-2	10.06	0.315	1.3	0.0023±0.0003	0.8202±0.0656	0.28±0.04
RD-3	10.37	0.839	1.8	bdl	0.4377±0.0350	bdl

Note: In addition to the otolith samples, barite ($BaSO_4$) (Ba-std), an elemental compound with structural characteristics similar to those of $CaCO_3$, was measured for ^{222}Rn leakage in the same manner as the otolith samples. bdl, below detection limit.

lutjanid otolith forms, new $CaCO_3$ deposition occurs equally on the dorso- and ventro-lateral planes rather than along the transverse plane. In contrast, sciaenid otoliths such as red drum accrue $CaCO_3$ more uniformly, resulting in an ellipsoidal shape (Fig. 5). The result is that red snapper otoliths are lower in weight but greater in surface area than red drum otoliths for most age-classes.

The significant difference in percent loss of ^{222}Rn between species was directly related to both the difference in ^{226}Ra activity and the surface area of otoliths. There appears to be conflicting views concerning the discrepancies in Rn loss across samples of similar structure. Rama and Moore (1984) studied the Rn transport mechanism from solids into groundwater and found that emanation behavior of ^{222}Rn remained unchanged after the outer surface of the medium was stripped away. Since ^{222}Rn contribution from the outside portion of the medium was not a contributing factor, they speculated that a significant portion of ^{222}Rn was being released from among the grains (Rama and Moore 1984). Krishnaswami and Seidemann (1988) compared ^{222}Rn and Ar leakage from various rocks and minerals and found that Ar leakage was barely detectable compared with the relatively high levels of ^{222}Rn loss (1.5–18.1%). They speculated that the high ^{222}Rn loss estimates were attributed to preferential enrichment of ^{226}Ra on grain surfaces or around grain boundaries. Through analyses of various natural and human-made materials, Moraswka and Phillips (1993) found that ^{222}Rn emanation was dependent on ^{226}Ra concentration and internal structure of the material. Regardless of the mechanism of Rn transport from within the solid, ^{222}Rn leakage was observed in all three studies.

The nature of Rn transport from within the otolith to the outside is still largely unknown, and we can only speculate on the mechanism by which loss occurs. For red snapper and red drum, our results support the notion that ^{226}Ra activity in the whole otoliths decreases with age (size) of the otolith and presumably decreased ^{226}Ra uptake. Whether induced environmentally or physiologically, our data also indicate that it is possible that less ^{226}Ra per gram is present in the outer layers of the otolith than in the core region. The mechanism by which ^{222}Rn emanation occurs can best be explained with two possible scenarios. First, if ^{222}Rn gas were emanating from within the whole otolith to the outside, thus averaging areas of low and high ^{226}Ra activities in the otolith, then ^{222}Rn loss estimates would be relatively low, as predicted in this study. On the other hand, if ^{222}Rn were only emanating from the outer layer of the otolith, then it is also possible that ^{222}Rn loss would be much higher than reported. Unfortunately, we were unable to calculate exact levels of ^{226}Ra in such a small amount of otolith material such as the outer layer; therefore, ^{226}Ra activities derived from whole otoliths were used. But even the highest ^{222}Rn emanation measurements coupled with the lowest ^{226}Ra activity measurements would produce an 11% loss of ^{222}Rn from the otolith. By incorporating these two scenarios, ^{222}Rn loss from otoliths would roughly be between 0 and 11%, in fact, similar to the loss estimates from other literature. Regardless of the scenario used, this loss of ^{222}Rn would be temporary (until the incorporated radium is buried deep enough to prevent loss upon decay). Therefore, the temporary loss may become insignificant with time and the use of cores may circumvent the problem. This notion appears to be valid, considering that in general, radiometric studies have produced age estimates similar to those of traditional aging techniques.

The observed ^{226}Ra activities within whole red snapper and red drum otoliths indicated that these fishes accumulated radionuclides at a rate inversely proportional to the otolith mass increase with age. Young fishes had significantly more ^{226}Ra per gram than did older fishes. A similar trend of decreasing ^{226}Ra activity with respect to increasing mean shell size was observed in mussels collected from the southern coast of Bangladesh (Alam et al. 1999). In the estuarine mussel *Modiolus striatulus* and the marine mussel *Perna viridis*, ^{226}Ra activity in shells ranged from 0.73 ± 0.32 to 0.50 ± 0.16 dpm·g^{-1} and from 0.86 ± 0.44 to 0.59 ± 0.19 dpm·g^{-1}, respectively. Our results indicated that different species occupying the same general location may have significantly different levels of ^{226}Ra per gram of otolith. The use of gamma analyses allowed for a reliable estimate of mean ^{226}Ra activity in a group of whole otoliths of the same size and age. In future studies, this mean ^{226}Ra activity per age group could be used as a reference to compare with the individual measurements of ^{226}Ra in otolith cores.

Red drum otoliths had a higher initial ^{226}Ra activity than red snapper otoliths of similar age, but both species displayed a decrease in ^{226}Ra activity with respect to age. This study demonstrated that ^{226}Ra is deposited onto the otolith at a rate nearly constant to that of Ca. Because red drum otoliths are generally larger at age than otoliths of red snapper, this would partially explain why the initial ^{226}Ra activities were higher. Red snapper exhibited a strong linear relationship between otolith weight and section age until approxi-

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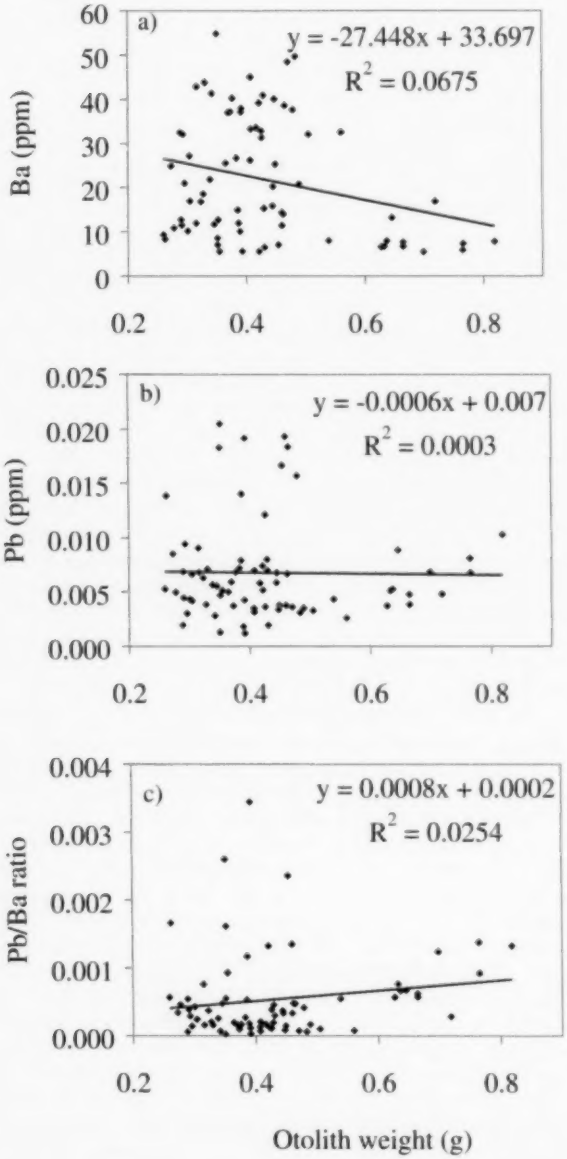
Fig. 4. (*a*) Ba, (*b*) Pb, and (*c*) the Pb/Ba ratio plotted against otolith weight for red drum from the northern Gulf of Mexico. The trend of decreasing Ba concentration with increasing otolith weight is actually an artifact of elemental differences by location (all otoliths >0.6 g were taken from Tampa Bay, Florida).



mately 10 years of age when the otolith growth slowed in relation to age. The otolith weight to age relationship could explain the initial high values of ^{226}Ra in young fish and the similar values of ^{226}Ra activity in 4-, 8-, and 15-year-old red snapper otoliths. In red drum, however, the relationship between otolith weight and ^{226}Ra activity was more linear through time. As with red snapper, the decrease in ^{226}Ra activity within red drum otoliths was inversely correlated with the increase in otolith mass with age.

A major change in habitat preference in adult red snapper and red drum is possibly another reason why young fishes (otoliths) had higher ^{226}Ra activities than did older fishes. Both red snapper and red drum exhibit a major shift in habitat type from the time when they are young (≤3 years) to the time of maturity, thus encountering variable in situ concentrations of ^{226}Ra. Juvenile red snapper are commonly found on the nearshore shrimping grounds (Render 1995). Subadults and adults are generally associated with offshore areas offering hard substrate such as natural reefs and oil and gas platforms (Render 1995). Juvenile red drum, however, occupy shallow estuaries and coastal bays that are continually flooded with nutrients from freshwater riverine systems. Adult red drum eventually move to nearshore waters to form large spawning aggregations during the late summer and early fall (Murphy and Taylor 1990). Fenton et al. (1990) found a similar trend in ^{226}Ra activity with age for blue grenadier (*Macruronus novaezelandiae*), a species known to exhibit a major change in habitat during its life cycle. Like red snapper, juvenile blue grenadier are commonly found in shallow coastal waters before maturing and gradually move offshore to the deeper waters of the continental shelf. Fenton et al. (1990) further reported that blue grenadier ^{226}Ra activity was greatest during the first few years of life and gradually decreased with the mass increase of the otolith. The subtle differences in habitat preference by species and life history stages may play a key role in ^{226}Ra accumulation.

The high ^{226}Ra activities observed in red snapper and red drum otoliths may be attributed to the high activities of ^{226}Ra being discharged from the Mississippi River. ^{226}Ra activity tends to be higher in the riverine and estuarine waters of the coastal shelf than in areas only a few kilometres offshore (Moore and Scott 1986). The high ^{226}Ra activities observed in red drum otoliths are the second highest only to Atlantic tarpon (*Megalops atlanticus*) collected from inshore Florida waters (Andrews et al. 1999*b*). The flux of ^{226}Ra in the lower Mississippi River mixing zone and in nearshore areas of the northern Gulf of Mexico has been examined in past studies (Moore and Scott 1986; Kraemer and Curwick 1991). The major sources of dissolved ^{226}Ra to marine waters including the northern Gulf of Mexico are from resuspension of bottom sediments and riverine input (Moore and Scott 1986). Throughout the world's oceans, natural concentrations of ^{226}Ra are generally higher in estuarine and nearshore waters than in shelf waters (Cochran 1982; Fenton et al. 1990). This concept is well illustrated in the northern Gulf of Mexico (Moore and Scott 1986). Behavior of ^{226}Ra in the Mississippi River mixing zone is strongly nonconservative and includes desorption similar to that reported for the Hudson, Pee Dee, and Amazon rivers (Moore and Scott 1986). In contrast, ^{226}Ra concentrations in the lower Mississippi River mixing zone are two to five times greater than in other rivers at the same salinity, mainly due to more suspended sediments in the water column (Moore and Scott 1986; Kraemer and Curwick 1991).

Stable elemental analyses of juvenile red drum otoliths indicated that the initial activity ratio of ^{210}Po to ^{226}Ra in this species was close to zero. Although low levels of Pb were present in most samples, the high amount of Ba (and presumably Ra) greatly reduced this ratio. West and Gauldie (1994) and Campana (1999) questioned the use of Ba as a

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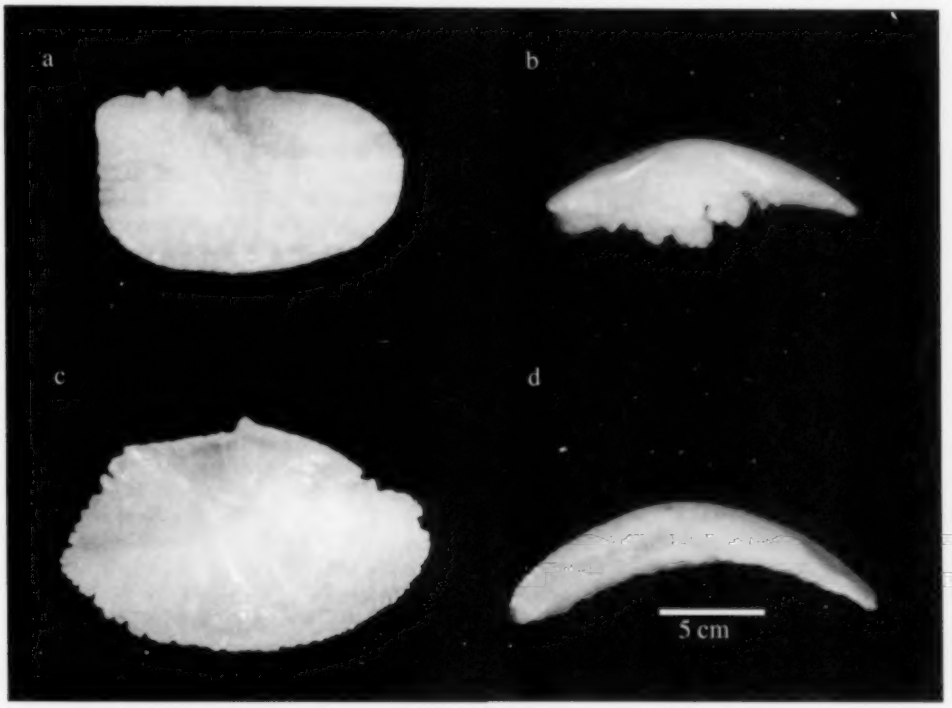
Fig. 5. Comparison of (*a* and *b*) 0.3741-g red drum and (*c* and *d*) 0.3828-g red snapper otolith as viewed from the top (left) and from the side (right). The red drum and red snapper otoliths in this figure were determined to be 1.3 and 2.9 years of age, respectively.



chemical analogue of Ra. The linear relationship between Ba and ^{226}Ra in the Atlantic and Pacific oceans indicated that both alkaline-earth elements are influenced by the same processes, i.e., particulate uptake at surface waters, gravitational settling, and release into the deep ocean (Chung 1980). In the northern Gulf of Mexico at several deep (>600 m) hydrocarbon seeps, analysis of mussel shells has shown that Ba increases logarithmically in relation to the linear uptake of ^{226}Ra, indicating a preferential biological uptake of Ba over Ra in shells (D.L. VanGent, unpublished data). Li and Chan (1979) compared the behavior of Ba and ^{226}Ra in the Hudson River estuary and also found a linear relationship, but only in the freshwater portion of the estuary. In more saline regions of the estuary, Ba decreased and ^{226}Ra increased linearly with salinity (Li and Chan 1979). Mean Ba concentrations in age-0 red snapper collected from the shallow shelf waters of the northern Gulf of Mexico were much lower (mean Ba concentration = 7.78 ppm, n = 69) than those observed in juvenile red drum collected from northern Gulf of Mexico estuaries (mean Ba concentration = 21.86 ppm, n = 74) (Patterson et al. 1998). Barium values of red drum from northern Gulf of Mexico estuaries were much higher than the mean value compiled from previous studies on estuarine species (mean Ba concentration = 8.2 ± 2.7 ppm, n = 3 studies) as well as freshwater and marine species (Campana 1999). While our results seem to support the concept that Pb and Ba can be used as stable proxies for ^{210}Pb and ^{226}Ra in red drum otoliths, assumptions associated with this approach may be more questionable than the assumptions of the radiometric method. Other than testing every sample for Pb, Ba, ^{210}Pb, and ^{226}Ra, the most effective approach would be wait to process the samples until after they are at least 2 years of age (from date of collection), thus allowing time for any ^{210}Po to come into secular equilibrium with ^{210}Pb (Andrews et al. 1999*a*).

The combined use of PERALS spectrometry and gamma detection equipment now allows researchers to test the assumptions concerning radiometric dating. The possibility of ^{222}Rn loss from otoliths can be considered for each species to which the radiometric dating technique is applied. The PERALS method of radioanalysis provided simple and accurate measurements of ^{222}Rn in (otolith) water samples and should be applicable to other estuarine and marine fishes. Nondestructive analyses of ^{226}Ra in pooled groups of otoliths using gamma spectroscopy was relatively straightforward; however, large quantities of whole otoliths by species and age group must be available. ^{226}Ra activities observed in pooled age-specific otolith samples of a species can be used as a guideline to estimate ^{226}Ra activity in otolith cores. Radiometric age validation of short- to long-lived marine fishes using PERALS and gamma spectroscopy holds great promise, provided that further research is undertaken to improve existing methodology.

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Stable isotope composition of walleye: ^{15}N accumulation with age and area-specific differences in $\delta^{13}C$

Nathanael C. Overman and Donna L. Parrish

Abstract: Stable nitrogen and carbon isotope ratios were measured for walleye (*Stizostedion vitreum*) collected across Lake Champlain, Vermont, to determine relationships between isotopic composition and diet, location of capture, length, weight, and age. Variation in $\delta^{13}C$ values reflected area-specific differences in isotopic composition of organisms collected across the lake. A critical assumption in the application of isotope techniques is that a predictable relationship exists between the diet and isotopic composition of an organism. Our results indicate that isotopic fractionation factors may not be independent of age as has largely been assumed. By combining stable nitrogen and carbon isotope analysis with conventional stomach content analysis, we documented significant age effects in the $\delta^{15}N$ composition of adult walleye that were not attributable to observed changes in diet. Age accounted for 81% of the variation in $\delta^{15}N$ values of walleye (ages 2–27, $N = 65$, $\delta^{15}N$ range = 15.3–19.2‰), providing evidence supporting ^{15}N accumulation over the life span of walleye. Therefore, the risk of making fauity inferences of trophic position and food web interactions based on $\delta^{15}N$ values may be increased when age is unknown. Our results indicate that metabolic effects associated with age require greater consideration in applications of stable isotope analysis.

Résumé : Nous avons mesuré les rapports des isotopes stables d'azote et de carbone chez des Dorés jaunes (*Stizostedion vitreum*) récoltés d'un bord à l'autre du lac Champlain, au Vermont, pour déterminer les relations entre la composition isotopique, d'une part, et le régime alimentaire, le site de capture, la longueur, la masse et l'âge, d'autre part. Les variations de $\delta^{13}C$ traduisent les différences spatiales dans la composition isotopique des organismes recueillis le long du transect. L'application des techniques isotopiques suppose nécessairement qu'on puisse prédire la relation qui existe entre le régime alimentaire et la composition isotopique d'un organisme. Nos résultats démontrent que les facteurs de fractionnement des isotopes ne sont peut-être pas indépendants de l'âge, comme on le suppose généralement. La combinaison d'analyses des isotopes stables d'azote et de carbone et d'analyses conventionnelles des contenus stomacaux tend à démontrer l'existence d'effets significatifs reliés à l'âge sur la composition du $\delta^{15}N$ chez les dorés adultes, effets qui ne s'expliquent pas par des changements perceptibles dans le régime alimentaire. L'âge explique 81% de la variation des valeurs de $\delta^{15}N$ chez les dorés (âges = 2–27; $N = 65$; étendue des $\delta^{15}N$ = 15,3–19,2‰), ce qui laisse croire qu'il y a accumulation de ^{15}N tout au cours de la vie du doré. En conséquence, le risque d'estimer faussement la position trophique et les interactions du réseau alimentaire basées sur les valeurs de ^{15}N augmente sans doute lorsque l'âge est inconnu. Les effets métaboliques associés à l'âge doivent donc être considérés avec plus de soin lors d'analyses des isotopes stables.

[Traduit par la Rédaction]

Introduction

Key to our understanding of ecosystem processes is the ability to accurately define trophic relationships (Paine 1988). This challenge is compounded in aquatic systems where trophic interactions can be difficult to determine using traditional methods such as stomach content analysis. Whereas diets can be useful, typical data provide only a "snapshot" of that period between ingestion and digestion and may not be an accurate method of describing long-term consumption patterns. In contrast, stable isotope analysis offers a broad picture of how nutritional sources are used by providing a time-integrated description of trophic relationships based on long-term assimilated diets. When combined

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with diets, stable isotope analysis can be a useful tool for verifying trophic interactions based on traditional methods.

Experiments on the influence of diet on the distribution of nitrogen and carbon isotopes in animals indicate that the isotopic composition of an organism is determined by the isotopic value of food consumed (DeNiro and Epstein 1978, 1981). Typically, $\delta^{15}N$ and $\delta^{13}C$ values of the consumer are heavier relative to dietary inputs because the lighter isotopes (^{14}N and ^{12}C) are either preferentially excreted (^{14}N) or respired (^{12}C). The retention of heavier isotopes with each increase in trophic level results in enrichment of approximately 3–5‰ between producer and consumer for nitrogen isotope ratios (DeNiro and Epstein 1981; Minagawa and Wada 1984; Peterson and Fry 1987) and 0–1‰ for carbon isotope ratios (DeNiro and Epstein 1978). The greater enrichment of nitrogen isotopes enables the use of nitrogen as an indicator of trophic position in a food web because isotopic values of predator and prey are easily distinguishable. For carbon isotopes, more limited enrichment allows carbon to be used as an indicator of the original source of carbon to the food web. Therefore, isotopic signatures for carbon are generally similar for both predator and prey and can be used to simplify relationships in complex food webs.

Walleye (*Stizostedion vitreum*) are long-lived top predators that, in Lake Champlain, Vermont exist almost exclusively as piscivores consuming a diet primarily of rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), white perch (*Morone americana*), and minnows (family Cyprinidae). Whereas stable isotopes depict time-integrated trophic relationships, dietary data provide a much finer taxonomic resolution and are necessary for validating isotope-based inferences. Despite the recent surge in stable isotope applications in aquatic ecosystems, few studies have incorporated both stable isotope and stomach content analyses (e.g., Gu et al. 1997; Beaudoin et al. 1999; Persson and Hansson 1999) in ecologically unique habitats of the same lake to investigate lake-wide differences in trophic ecology. Here, we combined both techniques for the purpose of determining trophic dynamics of walleye across oligotrophic, mesotrophic, and eutrophic habitats in Lake Champlain. However, in the course of this work, we discovered that a north–south gradient in $\delta^{13}C$ values was influencing walleye $\delta^{13}C$ values and that metabolic processes associated with age were possibly influencing walleye $\delta^{15}N$ values. The confounding influence of age limited our ability to determine dietary influence on the stable nitrogen isotope ratios of adult walleye. Stable isotope analysis has provided a powerful tool for aquatic ecologists studying food web ecology. However, our results suggest that the assumptions underlying dietary reconstruction from stable isotopes need further research.

Methods

Lake description

Lake Champlain forms the boundary between New York and Vermont and extends north into the Canadian province of Quebec. North to south, Lake Champlain extends 170 km, has a surface area of 1130 km^2, and has a volume of 26 km^3 (Levine et al. 1997). The lake's mean width is 6.61 km with a maximum width of 20.2 km (Meyer and Gruendling 1979). Whereas much of the lake is deep and near oligotrophic, the average depth in Lake Champlain is 19.4 m (Meyer and Gruendling 1979). At least 300 tributaries drain the 19 881-km^2 watershed surrounding Lake Champlain (Levine et al. 1997).

Morphology, habitat, and community structure vary considerably along Lake Champlain's extensive north–south border and between the areas of the lake under consideration in this study. To assure that a diversity of habitats and locations would be represented, four ecologically and geographically distinct lake regions were chosen as primary study areas: a mesotrophic area in the north (Inland Sea), a marginally oligotrophic area in the central lake body (Main Lake), a eutrophic area in the south (South Lake), and a transitional area between the eutrophic and oligotrophic areas (lower Main Lake).

Field and laboratory procedures

Walleye (ages 2–27, $N = 413$) were collected from May through November in 1997 and 1998. Sampling was focused in the Inland Sea and Main Lake during 1997 and in the South Lake and lower Main Lake during 1998. Experimental gill nets with mesh sizes ranging from 76- to 152-mm stretched mesh in 12.7-mm increments were fished overnight for approximately 12 h. Nets were 1.8 m deep and 106.7 m long, consisting of six randomized 15.2-m panels. To increase sample sizes, walleye were collected in gill nets set for 24 h in cooperation with the Vermont Department of Fish and Wildlife and from other nets set in the Inland Sea (Pientka 2000).

Samples used for isotope analysis were collected from July to November 1998. Because of limited sampling in the Inland Sea and Main Lake during 1998, all available walleye were used for isotopic analysis. For the lower Main Lake and South Lake, all walleye <500 mm and the largest and oldest individual from both areas were analyzed. Additional walleye were chosen to include walleye with identifiable prey species and to obtain a sample including a variety of lengths and weights. Prey fish were collected in beach seines and bottom trawls and unionid mussels (*Eliptio complanata*) were collected (June 1999) by hand via snorkeling or wading. Because lipids were not removed from samples prior to analysis, comparisons of $\delta^{13}C$ values were limited to the same species across areas to minimize error associated with different lipid levels among species.

Walleye stomach contents were obtained either by gastric lavage or stomachs were removed from whole fish frozen on dry ice during sampling. Total length, wet weight, second dorsal spine, and otoliths (from dead fish) were taken. Prey fish were counted, identified to species, and measured. Backbone lengths were recorded when digestion prohibited measurement of total or standard length, and vertebrae were counted when digestion prohibited visual identification to species. Prey with vertebrae counts in the 30s and having a skeletal structure resembling a minnow were classified as cyprinids (*Notropis* spp.). Prey lengths were converted to predigested wet weight via regressions. Relative importance (RI) of prey to walleye was determined according to the equation

$$RI = 100\ AI/\Sigma AI$$

$$AI = \%FO + \%N + \%WT$$

where AI is the absolute importance of prey in a designated prey taxon, %FO is the percentage of stomachs examined containing identifiable prey in a designated prey taxon, %N is the percentage of identifiable prey in a taxon relative to the total number of prey for all taxa, and %WT is the percentage of total wet weight of identifiable prey in all stomachs examined (George and Hadley 1979). This index was used to reduce the amount of bias associated with any one diet measure.

Interpreting trophic position relative to $\delta^{15}N$ values of primary consumers (such as unionid mussels) provides a more predictable

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measure of trophic position that adjusts for variation in baseline $\delta^{15}N$ values among systems (Cabana and Rasmussen 1996; Vander Zanden et al. 1997; Vander Zanden and Rasmussen 1999). We estimated trophic positions of both walleye and prey fish using the formula (Cabana and Rasmussen 1996)

$$\text{Trophic position} = [(\text{fish } \delta^{15}N - \text{mussel } \delta^{15}N)/3.4] + 2.$$

Because no unionids were collected in the lower Main Lake, trophic positions of lower Main Lake walleye were calculated using the average of the Main Lake and South Lake mean unionid $\delta^{15}N$ values.

Walleye were aged independently by at least two readers and assigned ages were corroborated by at least two separate readings. Dorsal spines were chosen for aging purposes to reduce the number of walleye killed; however, otoliths from walleye not released alive were used whenever available. Because dorsal spines underestimated ages of older walleye, dorsal spine age was converted to otolith age via linear regression, which resulted in dorsal spines aged 12 and older to be assigned to the next higher age.

At least 10 g of walleye muscle tissue was removed from the dorsal section between the head and dorsal fin for analysis. Fillets of large prey, whole bodies (gutted and beheaded) of small prey, and both adductor muscles from unionids mussels were washed well with deionized water, dried, and ground for analysis. A 10-mg (nitrogen analysis) and 3-mg (carbon analysis) sample of this material were taken from each animal for stable isotope analysis. Each sample was combusted at 900°C in a vacuum-sealed quartz tube containing 3 g of CuO wire, 2 g of granular Cu, and 500 mg of CaO (nitrogen analysis) or 600 mg of CuO wire and 500 mg of granular Cu (carbon analysis). Samples were analyzed in a dual-inlet mass spectrometer (model VG SIRA II) at the Environmental Stable Isotope Laboratory of the University of Vermont. Ratios of heavy to light stable isotopes were determined against internal N_2 and CO_2 standards (atmospheric N_2 for nitrogen and PeeDee Belemnite (PDB) limestone for carbon) and expressed in δ notation, defined as parts per thousand (‰) deviations from the standard

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is ^{15}N or ^{13}C and $R = {}^{15}N/{}^{14}N$ or $^{13}C/{}^{12}C$. More positive values are "enriched" relative to more negative or "depleted" values. Carbon isotope ratios of biological samples are usually depleted in ^{13}C relative to the standard and as a result have negative values. Analytical precision of the instrument over several years has been 0.2‰ for nitrogen and 0.1‰ for carbon.

Results

Stable nitrogen isotopes

Stable nitrogen isotope ratios determined for walleye, prey, and unionid mussels confirmed the piscivorous nature and trophic position of walleye as a top predator. Trophic positions in relation to baseline unionid $\delta^{15}N$ values ranged from 3.9 to 5.0 for walleye and from 3.3 to 3.6 for prey (Table 1). Lake-wide $\delta^{15}N$ values for walleye spanned 3.9 and 2.4–3.4‰ within a single area (Fig. 1). Values for prey spanned 2.1‰, with emerald shiner (*Notropis atherinoides*) exhibiting the lowest mean values and rainbow smelt the highest. Among prey, $\delta^{15}N$ values of rainbow smelt and white perch were higher than those of emerald shiner (analysis of variance (ANOVA), $F_{3,16} = 6.80$, $p < 0.05$; Tukey–Kramer). Unionid mussels were similar among lake areas with $\delta^{15}N$ values ranging from 8.4 to 9.7‰ (mean = 9.0).

Walleye $\delta^{15}N$ values were correlated with age, total length, and weight (Spearman rank order correlation, $p < 0.05$) in all areas with the exception of length and weight in the South Lake (Table 2). Across all areas, walleye $\delta^{15}N$ values increased moderately with length and weight (Spearman rank order correlation, range $r = 0.59$ for both, $p < 0.05$) but increased strongly with age (Spearman rank order correlation, lake-wide $r = 0.92$, $p < 0.001$). The stronger correlation of $\delta^{15}N$ values with age reflected the large variation in size at age characteristic of long-lived piscivores such as walleye. Overall, across all areas, $\delta^{15}N$ values increased significantly as a function of age (Fig. 2):

$$\log \delta^{15}N = 1.179 + (\log \text{age})(0.0743)$$

($N = 65$, $R^2 = 0.81$, $p < 0.001$). Inland Sea walleye were depleted in $\delta^{15}N$ values (ANOVA, $F_{3,61} = 7.90$, $p < 0.05$; Tukey–Kramer) when compared with those of other lake areas; however, Inland Sea walleye available for isotope analysis were limited to age 9 or younger. No differences in $\delta^{15}N$ values were evident among Main Lake, lower Main Lake, and South Lake areas.

Differences were marginal among walleye based on observed (including empty) stomach contents (ANOVA on ranks, $p = 0.07$). However, these results were confounded by differences in age. Means ranged from 16.1‰ for three walleye (mean age = 4) with contents containing cyprinids to 18.1‰ for seven walleye (mean age = 12) with white perch. For walleye containing rainbow smelt ($N = 21$), the mean $\delta^{15}N$ value was 17.3‰ and the mean age was 7.

Table 1. Mean trophic positions of walleye (categorized by age, total length, and area) and prey fish.

	N	Mean (±SD)	Range
Walleye			
Age group (years)			
2–4	22	4.18 (0.16)	3.87–4.49
5–7	9	4.41 (0.12)	4.22–4.60
8–10	11	4.63 (0.18)	4.22–4.93
11–15	8	4.73 (0.09)	4.58–4.86
16–20	10	4.82 (0.11)	4.66–4.99
21–27	5	4.89 (0.07)	4.82–4.97
Length-class (mm)			
350–499	13	4.18 (0.13)	3.98–4.45
500–549	12	4.33 (0.33)	3.87–4.95
550–599	21	4.68 (0.24)	4.23–4.99
>600	19	4.66 (0.16)	4.22–4.93
Area (age (years))			
Inland Sea (2–9)	18	4.27 (0.22)	3.87–4.75
Main Lake (4–20)	9	4.68 (0.25)	4.22–4.93
Lower Main Lake (2–27)	19	4.57 (0.33)	3.98–4.99
South Lake (2–25)	19	4.60 (0.25)	4.02–4.95
All areas (2–27)	65	4.51 (0.30)	3.87–4.99
Prey species (total length (mm))			
Rainbow smelt (103–138)	8	3.59 (0.11)	3.44–3.76
White perch (156–170)	3	3.55 (0.15)	3.37–3.64
Yellow perch (108–189)	6	3.34 (0.15)	3.10–3.55
Emerald shiner (75–86)	3	3.28 (0.10)	3.19–3.39

Note: Trophic position = [(fish $\delta^{15}N$ – mussel $\delta^{15}N$)/3.4)] + 2 (Cabana and Rasumssen 1996).

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Fig. 1. (*a*) $\delta^{15}N$ and (*b*) $\delta^{13}C$ of walleye, prey fish (RS, rainbow smelt; WP, white perch; YP, yellow perch; ES, emerald shiner), and unionid mussels (UM) collected in the Inland Sea (●), Main Lake (○), lower Main Lake (▼), and South Lake (▽) of Lake Champlain during 1998 and 1999.

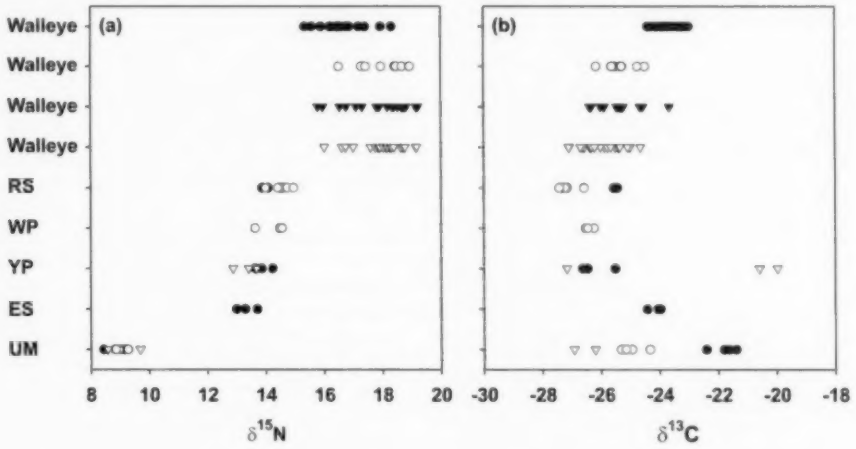

Table 2. Spearman rank order correlation coefficients (*p* values in parentheses) of age, total length, and weight of walleye and their $\delta^{15}N$ and $\delta^{13}C$ signatures in the Inland Sea, Main Lake, lower Main Lake, South Lake, and combined Lake Champlain areas.

		$\delta^{15}N$			$\delta^{13}C$		
	N	Age	Length	Weight	Age	Length	Weight
Inland Sea	18	0.67 (0.002)	0.64 (0.004)	0.72 (<0.001)	–0.13 (0.61)	–0.08 (0.74)	–0.20 (0.42)
Main Lake	9	0.73 (0.02)	0.68 (0.04)	0.73 (0.02)	0.35 (0.33)	0.33 (0.36)	0.40 (0.26)
Lower Main Lake	19	0.94 (<0.001)	0.51 (0.02)	0.53 (0.02)	0.15 (0.53)	0.02 (0.92)	–0.00 (0.72)
South Lake	19	0.94 (<0.001)	0.37 (0.11)	0.33 (0.17)	0.50 (0.03)	0.10 (0.68)	0.07 (0.78)
Lake Champlain	65	0.92 (<0.001)	0.59 (<0.001)	0.59 (<0.001)	–0.23 (0.06)	–0.09 (0.45)	–0.08 (0.53)

Note: Walleye were collected between July and November 1998.

Stable carbon isotopes

Analysis of stable carbon isotope ratios indicated that $\delta^{13}C$ values were different among walleye captured in each area, with the exception of values for walleye from the lower Main Lake that were not different from those for walleye from the Main Lake above or the South Lake below (ANOVA, $F_{3,61} = 48.34$, $p < 0.05$; Tukey–Kramer) (Fig. 2). Differences in $\delta^{13}C$ values among areas were especially pronounced for Inland Sea walleye where, aside from one lower Main Lake walleye (possibly a previous inhabitant of the Inland Sea), there was no overlap in $\delta^{13}C$ values with those for walleye from the rest of the lake. Across all areas, mean $\delta^{13}C$ values decreased from north to south in the lake. With the exception of two South Lake yellow perch, this north to south decrease in $\delta^{13}C$ values was evident for all biota analyzed (Fig. 1). The north–south gradient was most pronounced for unionid mussels for which no overlap in $\delta^{13}C$ values occurred between areas.

No relationships were evident between walleye $\delta^{13}C$ and age, length, or weight (Table 2) except for age in the South Lake (Spearman rank order correlation, $r = 0.50$, $p = 0.03$). Within the Inland Sea, lower Main Lake, and South Lake (ANOVA on ranks, $p > 0.05$) and the Main Lake (Mann–Whitney rank sum, $p > 0.05$), there were no differences in $\delta^{13}C$ values regardless of which prey species were consumed, including those with empty stomachs.

Diet analysis

Relative importance of prey determined for walleye of different age groups indicated that rainbow smelt by far were the most important prey for walleye as a whole (Table 3). This was especially evident in the Main Lake where aside from one large cisco (*Coregonus* spp.) consumed by an 18-year-old walleye, all Main Lake diets consisted of rainbow smelt. Inland Sea walleye also relied heavily on rainbow smelt across all age groups. In contrast with the Main Lake and Inland Sea, spiny-rayed species were most important in the South Lake. Whereas rainbow smelt became less important with age, the combined contribution of white perch and yellow perch to the diet was very important in all but the youngest age group. Diets in the lower Main Lake reflected the opportunity to use prey resources characteristic of both the Main Lake above and the South Lake below. White perch were especially important in the oldest age group but also contributed to diets of walleye as young as age 7. Stomach content analysis indicated that walleye consumed prey of similar range in size regardless of age (Fig. 3). For example, lake-wide ranges were 71–188 mm for prey consumed by age-5 walleye, 80–171 mm for age 10, 58–195 mm for age 15, and 85–201 mm for age 20. Ranges and (means) of prey species consumed were 67–201 mm (123) for rainbow smelt, 85–166 mm (135) for white perch, 110–185 mm (148) for yellow perch, and 25–176 mm (50) for cyprinids.

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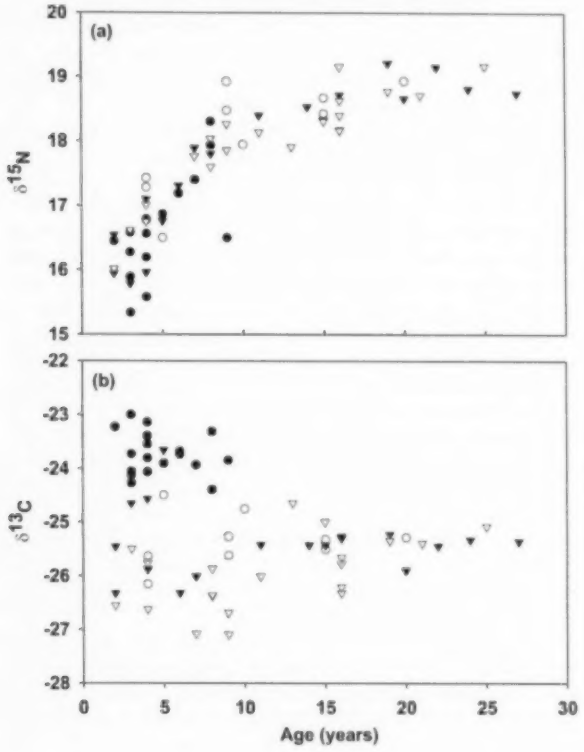
Fig. 2. (*a*) $\delta^{15}N$ and (*b*) $\delta^{13}C$ of walleye of ages 2–27 collected in the Inland Sea (●), Main Lake (○), lower Main Lake (▼), and South Lake (▽) of Lake Champlain during 1998.



Discussion

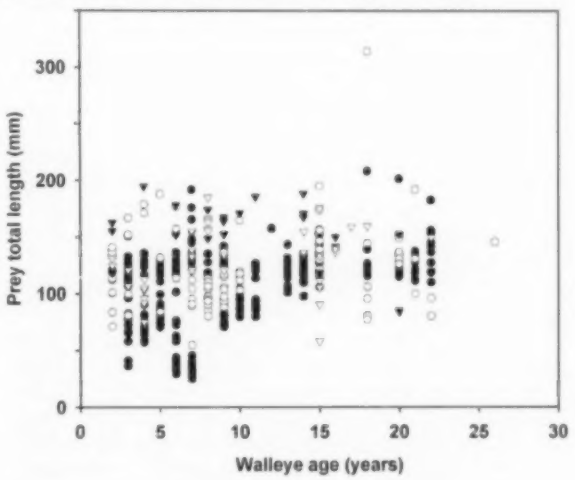
Positive relationships between $\delta^{15}N$ values and size have been determined for a variety of fish species including northern pike (*Esox lucius*) (Beaudoin et al. 1999), Eurasian perch (*Perca fluviatilis*) (Persson and Hansson 1999), Dover sole (*Microstomus pacificus*) (Rau et al. 1981; Spies et al. 1989), gizzard shad (*Dorosoma cepedianum*) and largemouth bass (*Micropterus salmoides*) (Gu et al. 1996), bowfin (*Amia calva*) and Florida gar (*Lepisosteus platyrhincus*) (Fry et al. 1999), and four fishes of the genera *Coregonus* and *Salvelinus* (Kline et al. 1998). Usually, strong positive relationships between $\delta^{15}N$ values and length, weight, or age have been interpreted as feeding at higher trophic levels. However, for adult walleye in Lake Champlain, the combined results of diet and stable isotope analyses provided evidence that walleye age was a better measure of $\delta^{15}N$ values than observed diet. Furthermore, the relationship between $\delta^{15}N$ values and age was consistent across a variety of ecologically unique habitats regardless of whether walleye ate soft-rayed or spiny-rayed species or a combination thereof. Although fewer rainbow smelt were eaten by older lower Main Lake and South Lake walleye, as was determined for rainbow smelt in Lake Ontario (Kiriluk et al. 1995) and other lakes in Canada (Vander Zanden et al. 1999), rainbow smelt $\delta^{15}N$ values were relatively enriched among prey species, a reflection of their piscivorous nature. Therefore, if anything, reduced consumption of rainbow smelt would be expected to decrease $\delta^{15}N$ values in older

Table 3. Relative importance index (RI) of prey fish of all age groups of walleye in the Inland Sea, Main Lake, lower Main Lake, and South Lake areas of Lake Champlain collected during 1997 and 1998.

	Age group (years)				
	2–4	5–7	8–10	11–15	≥16
Inland Sea					
Rainbow smelt	75.9	68.0	79.8	90.4	63.6
Cyprinidae	20.2	32.0	20.2	—	—
Yellow perch	3.9	—	—	9.6	30.0
Atlantic salmon	—	—	—	—	6.4
N	26	23	7	16	13
Main Lake					
Rainbow smelt	100	100	100	100	76.3
Cisco	—	—	—	—	23.7
N	8	6	5	3	8
Lower Main Lake					
Rainbow smelt	100	54.6	77.4	100	38.1
Cyprinidae	—	15.9	—	—	—
White perch	—	29.5	22.6	—	61.9
N	3	4	4	4	4
South Lake					
Rainbow smelt	73.3	42.6	18.6	—	18.9
Cyprinidae	11.5	—	—	33.0	—
White perch	—	57.4	41.3	67.0	59.1
Yellow perch	—	—	40.1	—	22.0
Gizzard shad	15.2	—	—	—	—
N	5	3	5	4	4

Note: *N* = number of walleye stomachs containing prey fish. RI = 100 AI/ΣAI and AI = %FO + %*N* + %WT.

Fig. 3. Walleye age and total length of all prey species in the stomachs of walleye collected in the Inland Sea (●), Main Lake (○), lower Main Lake (▼), and South Lake (▽) of Lake Champlain from May through November of 1997 and 1998.



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walleye. However, this was not the case. Possibly a decrease in consumption of rainbow smelt may have reduced the rate of increase in $\delta^{15}N$ values in older walleye from the lower Main Lake and South Lake, contributing to the asymptotic relationship between $\delta^{15}N$ values and age. Trophic position of top predators may vary in lakes with fewer opportunities to be piscivorous. If walleye were omnivorous, a wider range of $\delta^{15}N$ values would likely have masked the strong relationship between $\delta^{15}N$ values and age. For lake trout (*Salvelinus namaycush*), no relationship was evident between $\delta^{15}N$ values and age (Kiriluk et al. 1995) or trophic position and total length (Vander Zanden et al. 2000). Although lake trout undergo ontogenetic shifts in diet (Trippel and Beamish 1993; Madenjian et al. 1998), opportunistic feeding and omnivory prevented relationships with age from being detected. Even in the absence of omnivory, if no relationship exists between predator age (or size) and the size of prey consumed, ontogenetic diet shifts may not influence trophic position. Furthermore, if no relationship exists between prey body size and prey trophic position, as was determined for pelagic prey fish in lakes in Ontario and Quebec (Vander Zanden et al. 2000), shifting consumption to larger fish should not influence trophic position. Size ranges of prey consumed across Lake Champlain indicated that walleye were generally consuming the same range of prey sizes across ages.

With few exceptions, walleye in Lake Champlain were piscivorous, negating opportunistic feeding on multiple trophic levels as an explanation for increased $\delta^{15}N$ values with age. Switching from an invertebrate diet to piscivory can begin when walleye reach 30 mm total length (Mathias and Li 1982). Given that the half-time for metabolic replacement of nitrogen in mussel tissue is approximately 1 year (Hesslein et al. 1993), residual nitrogen from invertebrate feeding as young-of-the-year should be replaced by nitrogen from prey fish by the time walleye are adults. Furthermore, seasonal variation in the diets of walleye prey should be averaged in the isotopic composition of adult walleye, as should food consumed during temporary changes in habitat (e.g., spawning migrations).

Variation in diets across ages was not sufficient to explain the 3.9‰ increase in adult walleye $\delta^{15}N$ values with age, an increase suggesting that at least one trophic level difference in diets should exist between young and old walleye. This was especially true of Main Lake walleye, where diet data since 1984 corroborate the nearly exclusive diet of rainbow smelt. Instead, our results are consistent with metabolic or physiological parameters being an important determinant of $\delta^{15}N$ values. This may be particularly evident in the $\delta^{15}N$ composition of long-lived species, where heterotrophic nitrogen cycling occurs over a period of many years.

Isotopic fractionation has largely been assumed to be independent of the age of an organism (Hobson and Welch 1995); however, studies validating this conclusion are limited. Age did not influence isotopic fractionation in soft tissues of two marine mussels (Minagawa and Wada 1984), liver and kidney of cattle (Sutoh et al. 1987), or blood and feathers of adult falcons (Hobson and Clark 1992). Although metabolic effects associated with age were inferred from a positive relationship between $\delta^{15}N$ values and body mass in Dover sole (Rau et al. 1981), a nearly identical study attributed the same results to changing diet with size (Spies et al. 1989). Many isotopic studies of fish have limited analyses to length and weight, an approach that may be inadequate for long-lived species (such as walleye) where growth is highly variable. Whereas increased $\delta^{15}N$ values with fish size have often been observed, we are aware of no instances where this relationship has been demonstrated to result from ^{15}N accumulation (as opposed to replacement) in fish tissue with age. If ^{15}N accumulation is a lifelong metabolic effect, decreasing metabolic rates as walleye age may slow the rate of ^{15}N accumulation enough to produce an apparent plateau or asymptote at older ages.

In support of our results, small differences in the metabolism of ^{14}N and ^{15}N in amino acids from cultured rats were determined to occur during the transaminating process (Gaebler et al. 1966). Although positive relationships between $\delta^{15}N$ values and age were not evident for marine mussels, ^{15}N could be expected to accumulate in animals over time if nitrogen undergoes fractionation during the transamination or deamination process (Minagawa and Wada 1984). Indeed, isotopic fractionation has been determined to occur during transamination reactions, with the lighter isotope reacting faster in each direction of the reaction (Macko et al. 1986). Therefore, it is possible that ^{14}N is excreted from the body of some animals at a faster rate than is ^{15}N. Although other processes could counterbalance this net effect (Ponsard and Averbuch 1999), if total excretions are depleted compared with dietary intake, enrichment of the heavier isotope should occur over time.

Isotope values of top predators often exhibit larger ranges than expected. For instance, single-lake differences in $\delta^{15}N$ and $\delta^{13}C$ values were greater than 6‰ for lake trout in Lake Ontario (Kiriluk et al. 1995) and for northern pike, usually considered a specialist piscivore (Beaudoin et al. 1999). Isotopic signatures of Lake Champlain walleye demonstrated a lake-wide range of approximately 4‰ for both nitrogen and carbon isotopes. This variation was reduced, however, when walleye were separated into age groups ($\delta^{15}N$) and when separated by ecologically distinct lake areas ($\delta^{13}C$).

The lack of a relationship between $\delta^{13}C$ values and length, weight, and age in the Inland Sea, Main Lake, and lower Main Lake indicated that walleye of all ages within these areas were feeding on prey using the same carbon source. The small but significant positive relationship between age and $\delta^{13}C$ values in the South Lake may reflect a greater proportion of yellow perch and white perch consumed by older walleye. The two highly enriched South Lake yellow perch were only slightly longer (173 and 189 mm) than the average-size yellow perch (162 mm) consumed by South Lake walleye. It is unknown why these two yellow perch had such high $\delta^{13}C$ values. Values around –21‰ are typical of littoral invertebrates such as crayfish, whereas values near –27‰ are consistent with a phytoplankton and zooplankton based diet (Keough et al. 1996; Vander Zanden et al. 1998). For fish, organic carbon sources originate from phytoplankton, benthic algae, or plants, the relative importance of which is often system specific (Gu et al. 1996). In Lake Champlain, differences in $\delta^{13}C$ values among lake areas indicated that area-specific variation in $\delta^{13}C$ values must be considered for meaningful interpretation.

Habitat-related variation in stable isotope composition was determined for primary consumers among 14 lakes in On-

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tario and Quebec (Vander Zanden and Rasmussen 1999). For both $\delta^{13}C$ and $\delta^{15}N$, mean values decreased from littoral to pelagic to profundal habitats. Conversely, analysis of 29 fish species in Lake Okeechobee, Florida, indicated that $\delta^{13}C$ and $\delta^{15}N$ values generally increased from marsh habitat to offshore habitat. The marsh–offshore gradient was consistent with ontogenetic transitions from shallow to deeper water as fish grew (Fry et al. 1999). In Lake Champlain, the north–south gradient in $\delta^{13}C$ values for most organisms suggested that stable isotope analysis might be a useful tool for comparing physical and chemical properties across the lake.

Similarly, stable isotope analysis may provide a useful method of monitoring watershed influences. For instance, variation in the $\delta^{15}N$ values of primary consumers (such as unionid mussels) was determined to be an indicator of human population density within a watershed because $\delta^{15}N$ values at the base of the food web reflected relative amounts of anthropogenic nitrogen (Cabana and Rasmussen 1996). Of the 40 lakes studied, mean values for unionids ranged from 1.2 to 9.0‰, with more heavily influenced lakes having higher values. Unionids averaged 9.0‰ in Lake Champlain where most of the nitrogen-rich runoff entering the lake is agricultural in origin (Levine et al. 1997) and can be traced to livestock operations.

High $\delta^{15}N$ values at the base of the food chain would also explain why Lake Champlain walleye were considerably more enriched in $\delta^{15}N$ (15.3–19.2‰) than those from Lake Superior (9.4–10.8‰) (Keough et al. 1996) or from lakes in Ontario and Quebec (approximately 11–14.5‰) (Cabana and Rasmussen 1996). Although walleye were relatively enriched in $\delta^{15}N$, trophic positions of adult walleye in Lake Champlain (range 3.9–5.0) were roughly equivalent to the range of trophic positions determined by baseline-corrected $\delta^{15}N$ values for walleye from lakes in Ontario and Quebec (Vander Zanden et al. 1997). $\delta^{15}N$ values for walleye from lakes in Ontario and Quebec averaged 8‰ higher than for unionid mussels (Cabana and Rasmussen 1996), which was similar to an enrichment of 8.6‰ above unionids in Lake Champlain.

A review of stable isotope techniques in animal ecology concluded that interpretation of stable isotope data relies on untested and generally unrecognized assumptions and that failure to recognize these assumptions can lead to a large body of data that are subject to misinterpretation (Gannes et al. 1997). Furthermore, progress in the use and interpretation of stable isotope applications in animal ecology will be limited unless field data are accompanied by laboratory experiments. Although the results of this study indicate that age is not independent of $\delta^{15}N$, the only way to determine unequivocally if ^{15}N accumulation occurs with age in fish is to raise them on isotopically homogenous diets through time under laboratory conditions. Stable isotope techniques have been effective for trophic analysis and dietary reconstruction in a number of systems (e.g., Hesslein et al. 1991; Hobson and Welch 1995; Vander Zanden et al. 1998). However, unexpected or unexplained variation in isotopic composition of some aquatic organisms is not uncommon (e.g., Hesslein et al. 1991).

In conclusion, our results demonstrate that age-related variation in $\delta^{15}N$ values and area-specific variation in $\delta^{13}C$ values are important considerations in stable isotope analysis. Results of carbon isotope analysis are especially applicable to lake management because $\delta^{13}C$ values have potential for use in determining migratory patterns and monitoring within-lake habitat use. Results of stable nitrogen isotopes are of particular importance for trophic analysis of long-lived species where ignoring age could result in misinterpretation of trophic position or faulty diet reconstruction. $\delta^{15}N$ values in muscle tissue increase log-linearly throughout the life span of walleye in Lake Champlain; consequently, the use of nitrogen isotopes as a tracer for dietary analysis and determining trophic positions of some long-lived, slow-growing, piscivorous fish may need to be reevaluated. These results do not invalidate dietary reconstructions based on stable isotopes; however, our results suggest that either a species-specific correction factor is needed to account for the rate of ^{15}N accumulation with age or isotope-based interpretations of diet should be limited to individuals of similar age. In the meantime, age- and metabolic-related accumulation of ^{15}N needs to be investigated further, and estimates of trophic position based solely on $\delta^{15}N$ values should be interpreted cautiously when estimates of age are unknown.

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